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STUDIES IN CALCIUM AND PHOSPHORUS METABOLISM*

XI. THE CALCULATION OF ACID BASE CONTENT OF THE DIET

By

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THE estimation of the acid base balance in diets is becoming progressively more important in metabolic studies as well as in the treatment of disease. It is obvious that in the study of electrolyte metabolism there are several important factors—the intake, the absorption from the intestinal tract, and the effect of absorption upon the excretion both of recently ingested and of previously stored electrolytes. Thus it is important to determine quantitatively the effect of the ingestion of excess acids upon the excretion of bases such as calcium, which are stored in the body.

A series of experiments in such problems was undertaken in the metabolic ward previously described by Bauer and Aub (1). The results of these observations on the electrolyte metabolism of man are reported elsewhere, but a discussion of the difficulties involved in the accurate dietetic management of these investigations is the purpose of this communication. In these observations the attempt was made to vary the potential acidity[1] or alkalinity of the diet without altering the total low calcium intake. The calcium and ammonia excretion then afforded an accurate method of determining the influence of varying procedures upon the excretion of fixed electrolytes stored in the body.

In 1912 Sherman and Gettler (2) called attention to the importance of inorganic constituents of the diet as sources of the excess acid or base excreted, and as important factors influencing the amount of urinary am-

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[1] Potential acidity may be briefly defined as the excess acid (referred to the hydrogen ion concentration of the blood) of the inorganic residuum of the diet after oxidation.

monia. Tables of analyses for many foods have since appeared in Sherman's book from which the excess acid or base content of the common articles of the dietary may be estimated.

Subsequently, Blatherwick (3) confirmed Sherman's deductions, that, with the exception of certain fruits yielding organic acids, the alkalinity or acidity of the urine is normally determined by the inorganic constituents of the food. The relation between acid excretion and calcium excretion has been discussed by Aub (4) and associates (5).

For the purpose of investigating accurately the excretion of inorganic electrolytes on diets of known composition, it is necessary to control the potential acidity of the food in order to arrive at sufficiently accurate standard levels of excretion. If the inorganic constituents of a diet are known quantitatively and if it be assumed that no considerable amount of organic acid escapes oxidation, it is possible to calculate approximately the potential acidity or alkalinity of a given dietary. The calculation of the potential acidity of a diet can be made if the inorganic base-forming elements, *i.e.* Ca, Mg, Na, and K, be summated in terms of N alkali and the inorganic acid-forming elements, *i.e.* Cl, S, P, be summated in terms of N acid.[2] The difference between these two sums gives the potential acidity or alkalinity of the diet, barring the excretion of much organic acid. That this calculation cannot be made with great accuracy, even when food is accurately weighed, is apparent not only from consideration of possible natural daily variations in the composition of foodstuffs, but also from the difference in values to be found in the literature for individual foods. Most of the estimations of the inorganic constituents of the foodstuffs used have been based on tables compiled by Sherman (6). If the data and discussion which follow indicate discrepancies between estimated values and actual analyses of our diets, they should in no sense be interpreted as unfavorable criticism of Sherman's very valuable work. These differences do not detract from the value of such compilations for use in routine dietetics. It is our purpose merely to examine the reliability of estimated potential acidity data for accurate metabolic experiments in the clinic. In some instances the earlier values published in 1911 by Sherman, and long used by dietitians, are given for comparison with his more recent estimates to emphasize the necessity of using up-to-date tables in dietary computations.

The following estimate for meat, based on Sherman's 1927 figures for meat protein (see Table I), gives an excess acid of 16.5 cc. N acid per 100

[2] Sulfur is reckoned as divalent; and the valence of phosphorus considered to be approximately 2.

grams. His 1911 figures, however, yield values for several different samples of meat, as follows: 13.91, 10.05, 12.00, and 13.67 cc. N (averaging 12.4 cc. N. acid).

TABLE I

POTENTIAL ACIDITY OF MEAT

Meat (21.3% protein)	Ca		Mg	K	Na	P		Cl	S
Total, gm.	.012	*.008*	0.025	0.361	0.090	0.230	*0.172*	0.081	0.244
Total, cc. N	.62	*.40*	2.1	9.2	3.9	14.8	*11.1*	2.3	15.2
Total cc. N acid						32.3	*28.6*		
Total cc. N base						15.8	*15.6*		
Total cc. N excess acid						16.5	*13.0*		

Note: Supplementary analyses from Massachusetts General Hospital of Ca & P are indicated by italics. Other values are taken from Sherman, 1927.

Table II shows that the difference between Sherman's estimate of excess base in apples in 1911 and 1927 is negligible.

TABLE II

APPLE, ACCORDING TO SHERMAN (per 100 grams)

	Ca		Mg	K		Na		P		Cl		S	
Total, gm.	.007	*.010*	.008	0.127	*0.125*	.011	*.015*	.012	*.013*	.005	*.004*	.006	*.005*
Total, cc. N	.4	*.5*	.7	3.3	*3.2*	.5	*.7*	.8	*.8*	.1	*.1*	.4	*.3*
Total cc. N base								4.9	*5.1*				
Total cc. N acid								1.3	*1.2*				
Total cc. N excess base								3.6	*3.9*				

Note: Figures in italics according to Sherman, 1911. Other figures according to Sherman, 1927.

In Table III, however, is given a similar estimate for banana calculated from Sherman's 1927 figures. The 1911 estimate (in italics) was 5.8 cc. N base per 100 grams edible portion as compared with 8.5 in 1927.

Analyses of several foods for calcium and phosphorus content have been made in this laboratory, some of which are compared (in Table IV)[3] with the corresponding values given by Sherman. Substitution of these values for Sherman's will yield a different acid base value.

[3] A more complete table of analyses made in this laboratory appeared in the *Journal of the American Dietetic Association*, Vol. III, No. 2, September, 1927. The phosphorus determinations are open to the objection that the samples were ashed in a furnace. Inasmuch as the results are frequently higher than Sherman's figures, however, the objection would appear not to be consistently valid.

TABLE III
BANANA, ACCORDING TO SHERMAN
(per 100 grams)

	Ca		Mg	K	Na	P	Cl	S
Total, gm.	0.009	*.007*	0.028 *0.024*	0.401 *0.415*	0.034 *0.015*	0.031 *0.024*	0.125 *0.200*	0.010 *0.013*
Total, cc. N	.5	*.4*	2.3 *2.0*	10.3 *10.6*	1.5 *.7*	2.0 *1.5*	3.5 *5.6*	.6 *.8*
Total, cc. N base			14.6 *13.7*					
" " " acid			6.1 *7.9*					
" " " excess base			8.5 *5.8*					

Note: Figures in italics according to Sherman, 1911. Other figures according to Sherman, 1927.

In Table V are given the acid base values for a few foodstuffs, calculated according to Sherman's figures and again with values for Ca and P from this laboratory substituted for his 1927 figures. An extreme example is found in chicken, which, according to Sherman (1911), contained, per 100 grams, excess acid amounting to 17.0 cc. N acid, but according to our analysis only 10.3 cc. N acid. Inasmuch as chicken may constitute an important part of a high acid or high protein diet, the discrepancy between these estimates is of considerable importance.

Another food for which it is rather difficult to evaluate the acid base content is cooked bacon, due to the fact that in the process of cooking much fat separates from the food, leaving behind a high salt content relative to the percentage weight. The difference in the potential acidity of bacon, before and after cooking, is illustrated by Table VI. The estimates for cooked bacon are based on analyses for Ca and P performed in the Massachusetts General Hospital. The NaCl content was determined by Miss Phoebe E. Drury at the Collis P. Huntington Memorial Hospital by Van Slyke's method. The protein content was taken as 23 per cent (Sherman, 1927, cooked bacon). Other figures are fixed as twice those for the mineral content of raw bacon.

The discrepancy in total electrolyte content is of course far greater than the difference between the values for potential acidity. Nevertheless, for equal

TABLE IV

SHERMAN'S CA AND P FIGURES COMPARED WITH M.G.H.* ANALYSES

	Calcium (per 100 grams of edible portion)		Phosphorus (per 100 grams of edible portion)	
	Sherman	M.G.H.	Sherman	M.G.H.
Apple	.007	.010	.012	.018
Banana	.009	.007	.031	.042
Corn	.006	.005	.103	.061
Steak	.012	.008	.229	.172
Chicken	.011 (1911)	.018	.258	.162
Bacon	.006	.030	.108	.096

* Massachusetts General Hospital.

TABLE V

COMPARISON OF ACID BASE VALUES CALCULATED FROM DIFFERENT DATA

	Sherman, 1911			Sherman, 1927		Ca and P from M.G.H. with Sherman, 1927	
	Base cc. N	Acid cc. N		Base cc. N	Acid cc. N	Base cc. N	Acid cc. N
Bread	—	—		—	6.5	—	6.6
Apple	3.8	—		3.5	—	3.3	—
Banana	5.6	—		8.3	—	7.5	—
Steak	—	13.9 10.1 12.0 13.7	Av. 12.8	—	16.5	—	13.0
Chicken	—	17.0		—	—	—	10.3 (Sherman, 1911)

weights of protein, the calculated acidity for raw bacon would be nearly twice that for cooked. The problem is complicated not only by the difference in analytical values obtained from different sources for the same food, but also by the difference in salt content of various brands of bacon. Actually, the raw bacon obtained in the Massachusetts General Hospital showed on analysis 43 cc. of normal chloride solution per 100 grams.

The difficulty could be simplified in large measure if patients would eat all the bacon fat eliminated during cooking. In practice, however, it is not easy to persuade patients to do this. Bacon, therefore, is probably best

TABLE VI
ESTIMATED EXCESS ACID IN BACON, RAW AND COOKED
(Per 100 grams)

	Ca		Mg		K		Na		P		Cl		S	
Total, gm.	0.006	*0.030*	0.012	*.023*	0.169	*0.336*	0.042	*2.7*	0.108	*0.096*	0.038	*4.1*	0.115	*0.229*
Total, cc. N	0.3	*1.5*	1.0	*1.9*	4.3	*8.6*	1.8	*116.5*	7.0	*6.2*	1.1	*116.5*	7.2	*14.3*

Total, cc. N acid	15.3	*137.0*
" " " base	7.4	*128.5*
" " " excess acid	7.9	*8.5*

Note: Figures in italics are for cooked bacon. Other figures represent raw bacon according to Sherman, 1927.

avoided even though it is an excellent constituent for a high-protein, high-caloric, low-calcium diet.

In Table VII is shown a neutral diet. This is more basic than originally intended, for the original estimate based on Sherman's 1911 figures with certain amplifications from this laboratory was 0.1 cc. N acid.

The synthesis of a low calcium diet high in potential acidity is accomplished largely by feeding a maximum of protein, together with cereal products (such as rice and bread); eliminating vegetables (such as potato) or fruits which are potentially alkaline. That the precise excess acid value of such a diet is difficult to determine can be seen from the variation in estimated acidity for steak and chicken already commented upon.

In Table VIII is shown the variation in a very acid diet calculated from two sets of data. This diet constitutes as acid a diet as could be arranged without recourse to foods (like cranberries) yielding a high organic-acid excretion. The variation is approximately 20.0 cc. of N acid per day, which is almost altogether due to the phosphate analyses. It is interesting to note that our original estimate was 72.3 cc. N acid.

In Table IX another similar high acid diet is given, formerly presumed to yield 68.9 cc. N excess acid on the basis of Sherman's 1911 figures with our Ca and P analyses. Calculated from Sherman's 1927 figures plus Massachusetts General Hospital Ca and P this yielded 65.2 cc.

TABLE VII
NEUTRAL DIET

	Gm.	Ca		Mg	K	Na		P		Cl		S
Bread	150	0.040	*0.017*	0.034	0.162	0.591	*0.510*	0.139	*0.122*	0.911	*0.786*	0.157
Butter fat	40											
Bacon*	30	.003	*.009*	.007	.101	.025	*.804*	.064	*.028*	.022	*1.239*	.069
Apple	200	.014	*.020*	.016	.254	.022	.022	.024	*.036*	.010	.010	.012
Sugar	60											
Steak	100	.012	*.008*	.025	.360	.089	.089	.226	*.172*	.080	.080	.244
Potato	250	.035	*.027*	.070	1.072	.053	.053	.145	*.117*	.095	.095	.075
Corn	100	.006	*.005*	.033	.113	.040	.040	.103	*.061*	.014	.014	.046
Banana	100	.009	*.007*	.028	.401	.034	.034	.031	*.042*	.125	.125	.010
Honey	30	.001	.001	.005	.115			.005	.005	.008	.008	
Chicken**	45	.005	*.007*	.016	.209	.042	.042	.115	*.073*	.027	.027	.131
Tomato	125	.014	*.008*	.012	.343	.012	.012	.032	*.068*	.043	.043	.017
Ginger ale	400	.002	.002					.004	.004			
Total, gm.		.141	*.111*	.246	3.130	.918	*1.606*	.888	*.728*	1.335	*2.427*	.761
Total, cc. N		7.00	*5.50*	20.2	80.0	*39.9*	*69.9*	57.2	*46.9*	37.6	*68.4*	47.4

Total, cc. N base	147.1	*175.6*
" " " acid	142.2	*162.7*
" " " excess base	4.9	*12.9*

Note: Supplementary analyses from M.G.H. Ca & P determinations are indicated by italics. Other values are taken from Sherman, 1927.

* Figures in italics represent Ca, P, and NaCl analyses from this laboratory.

** Figures in italics represent analyses from Sherman, 1911, plus M.G.H. Ca & P.

TABLE VIII
HIGH ACID DIET

	Gm	Ca		Mg	K	Na		P		Cl		S
Bread	300	.081	*.033*	.069	.324	1.182	*1.020*	.279	*.243*	1.821	*1.572*	.315
Bacon*	45	.005	*.013*	.010	.152	.037	*1.206*	.097	*.043*	.034	*1.858*	.103
Steak	200	.024	*.016*	.050	.720	.178	.178	.458	*.344*	.160	.160	.488
Corn	200	.012	*.010*	.066	.226	.080	.080	.206	*.122*	.028	.028	.092
Rice (Cooked Wt.)	100	.003	*.006*	.012	.027	.009	.009	.036	*.075*	.020	.020	.045
Chicken** (dark meat)	60	.006	*.012*	.022	.279	.057	.057	.154	*.094*	.036	.036	.172
Macaroni (raw wt.)	30	.006	*.005*	.011	.039	.002	.002	.043	*.039*	.021	.021	.051
Sugar	30											
Butter fat	40											
Ginger ale	400	.002	.002					.004	.004			
Total, gm.		.139	*.097*	.240	1.767	1.545	*2.552*	1.277	*.964*	2.120	*3.695*	1.266
Total, cc. N		7.0	*4.8*	19.7	45.2	67.2	*111.0*	82.2	*62.1*	59.8	*104.2*	79.1

Total, cc. N acid	221.1	*245.4*
" " " base	139.1	*180.7*
" " " excess acid	82.0	*64.7*

Note: Supplementary analyses from M.G.H. Ca and P determinations are indicated by italics. Other values are taken from Sherman, 1927.

* Figures in italics represent Ca, P, and NaCl analyses from this laboratory.

** Figures in italics represent analyses from Sherman, 1911, plus M.G.H. Ca & P.

TABLE IX
HIGH ACID DIET

	Gm.	Ca		Mg	K	Na		P		Cl		S
Bread	300	0.081	*0.033*	0.069	0.324	1.182	*1.020*	0.279	*.243*	1.821	*1.572*	.315
Bacon*	45	.005	*.013*	.010	.152	.038	*1.206*	.096	*.043*	.034	*1.858*	.103
Steak	200	.024	*.016*	.050	.720	.178	.178	.458	*.344*	.160	.160	.488
Rice	100	.003	*.006*	.012	.027	.009	.009	.036	*.075*	.020	.020	.045
Chicken**	60	.006	*.012*	.022	.278	.057	.057	.154	*.094*	.036	.036	.172
Macaroni	30	.006	*.005*	.011	.039	.002	.002	.043	*.039*	.021	.021	.051
Sugar	70											
Butter fat	30											
Ginger ale	400	.002	.002					.004	.004			
Fudge (low Ca)	20			.002	.004			.003	.003			
Total, gm.		.127	*.087*	.176	1.544	1.466	*2.472*	1.073	*.845*	2.092	*3.667*	1.174
Total, cc. N		6.4	*4.4*	14.4	39.4	63.7	*107.6*	62.0	*54.4*	58.9	*103.4*	73.2

Total, cc. N acid	194.1	*231.0*
" " " base	123.9	*165.8*
" " " excess acid	70.2	*65.2*

Note: Supplementary analyses from M.G.H. Ca & P determinations are indicated by italics. Other values are taken from Sherman, 1927.

* Figures in italics represent Ca, P, and NaCl analyses from this laboratory.

** Figures in italics represent analyses from Sherman, 1911, plus M.G.H. Ca & P.

of N acid. Using Sherman's 1927 figures alone the result would be 70.2 cc. N acid.

Inasmuch as it was desirable for certain purposes to keep the calcium content of our diets at a minimal level of about 100 mg. of calcium per day, it was not found feasible to construct adequate diets more alkaline than 40 cc. of N base per day without exceeding this limit. With a high calcium content it is not hard to exceed 150 cc. of N base daily on an adequate diet (with sufficient protein).[4] In our experiments, however, it was necessary to rely upon additions of sodium bicarbonate and sodium citrate to produce extreme alkalinity.

The following table (X) illustrates the most alkaline mixed diet, adequate in protein but low in calcium, that we could devise for a patient. The excess base amounts to only 30 cc. N alkali.

TABLE X
LOW CALCIUM MAXIMUM BASIC DIET

	Grams Daily	Acid cc. N	Base cc. N	Protein Grams	Calcium Grams
Apple	150		5.64	.45	.015
Crackers	60	4.68		5.88	.013
Bacon	30	2.52		6.9	.009
Potato	500		31.70	9.5	.055
Banana	250		13.9	2.75	.017
Steak	100	12.8		21.3	.008
		20.0	51.2	46.8	.117

Total cc. N excess base—31.2

DISCUSSION

It is evident that an error of 10 cc. or more of N acid may commonly be encountered in calculating the potential acidity of a daily diet according to the data employed. The production of organic acids is another uncertain factor in computing an acid base balance. In view of the uncertainty, however, which attends bowel activity (absorption, intestinal rate, excretion) and which is in large measure uncontrolled, such calculations afford an adequate basis at least for interpreting changes in excretion associated with quantitated variations from a constantly continued "control" diet.

In any case, if the actual weighed dietary records be preserved, they may always be recalculated in the light of further analytical knowledge.

[4] See Sansum, W. D., Blatherwick, N. R., and Smith, F. H., *Journal of the American Medical Association*, LXXXI, 883, 1923. These authors present an alkaline diet qualitatively, but say nothing as to its total excess base content.

The difficulties of calculating exactly acid base balance can be simplified greatly if the diet consist of only a very few simple ingredients. This procedure, however, is not practicable with patients over a long period of study. In this series of studies patients were given a simple mixed diet which was maintained uniform, as nearly as possible, in order to determine the effect of quantitative variations in simple foods.

Analysis of Specimen Diets

In view of the difficulties already described in calculating the potential acidity of diets, it seemed desirable to ascertain this value directly for comparison with the calculated values. It was at first intended that this should be done by combustion of a sample in a calorimetric bomb; but it

Table XI
Comparison Between Analyses and Estimated Content of Daily Diet

	Diet I Neutral		Diet II Neutral		Diet III Acid		Diet IV Acid		Diet V Basic	
	Calc'd cc. N	Found cc. N	Calc'd cc. N	Found cc. N	Calc'd cc. N	Found cc. N	Calc'd cc. N	Found cc. N	Calc'd cc. N	Found cc. N
Phosphorus	49.0	41.9	57.2	41.4	82.0	56.4	69.0	55.5	47.1	35.2
Chlorine	70.2	68.0	72.0	75.9	111.0	116.2	111.0	92.2	66.9	63.4
Sulfur	39.0	27.9	47.2	46.7	79.0	71.6	81.4	53.8	35.8	34.5
Total acid	158.2	137.8	176.4	164.0	272.0	244.2	261.4	201.5	149.8	133.1
Total base	171.2	124.8	180.6	159.5	189.8	177.4	174.6	147.5	190.3	159.0
Net acidity	−13.0	+13.0	−4.2	+4.5	+82.2	+66.8	+86.8	+54.0	−40.5	−25.8
Difference	26.0		8.7		15.4		32.8		14.7	

was pointed out to us by Doctors Francis G. Benedict and H. C. Sherman that the inconstant formation of nitric acid under such conditions would lead to gross inaccuracy until a suitable technique might be evolved. For the time being, therefore, it was necessary to be content with individual analyses of the total base and the acidic elements of one day's diet which, summated, could be compared with the calculated values.[5] The results ob-

[5] The entire diet for one day was finely ground, mixed thoroughly, dried, and powdered. The analyses were made both on samples of powdered dry diet and on nitric acid extracts of diets. Fiske's methods for total base, phosphate, and total sulfur were employed, and Van Slyke's for chloride. We are indebted to Miss Dorothy M. Tibbetts and Miss Phoebe E. Drury for these results.

tained from five typical diets are recorded in Table XI in which the theoretical estimates were obtained from Sherman (1927) supplemented by our analysis of cooked bacon.

It is apparent that the potential acidity as calculated is not very accurate and that deviations from neutrality were actually much less marked than anticipated.

It has become progressively more obvious during these studies upon inorganic salts that variations should only be made very slowly as not infrequently a week elapses before the patient has attained a steady state after a variation in diet. Many of the results in the literature are, therefore, of little value because they represent only transitional states due to the brevity of observation.

The complexity of metabolic processes occurring within the body, moreover, is such that marked changes in the diet may result in only moderate variation in excretion levels (7).

The urinary response to such diet is indicated by a few instances in Table XII, from which it will be observed that the response to change in diet is significant only in a qualitative sense. The first of these patients had tetany (due to poor fat assimilation), and the last two were essentially normal.

TABLE XII

POTENTIAL ACIDITY OF DIET VS. ACIDITY OF DAILY URINE (Average)

	Estimated acidity of diet cc. N		Titratable acidity $-CO_2$ of urine cc. N	Urinary ammonia cc. N	Total acid excretion cc. N
	Calc'd	Found			
DeBa. neutral	−13.0	+13.0	−5.5	60.6	55.1
Da. neutral	−4.2	+4.5	16.7	24.1	40.8
Da. high acid	+82.2	+66.8	18.2	45.8	64.0
An. neutral	+1.0		−20.9	38.9	18.0
An. high acid	+86.8	+54.0	−19.5	104.9	85.4

There is obviously much to be desired in improvement of the calculation of potential acidity in diets. For careful metabolic work it is apparently imperative either to analyze each diet as fed, or to feed extremely simple

foodstuffs. It is at least essential to employ a constant basal diet to which potentially acidic or basic additions may be made.

SUMMARY

1. The calculation of the potential acidity or alkalinity of diets is discussed.

2. Results so estimated with the help of standard nutrition tables are compared with actual analyses and with the urinary acid excretion on feeding such diets.

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SOME FACTORS AFFECTING ACCURACY IN THE COLLECTION OF DATA ON THE GROWTH IN WEIGHT OF SCHOOL CHILDREN*

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A STUDY on the growth in height and weight of Texas school children is being conducted by the Division of Rural Home Research of the Texas Agricultural Experiment Station in cooperation with the public schools of San Antonio. The elementary supervisors selected three schools to participate in this project. Each school provides a representative population of from 500 to 600 of one of three racial groups, white, Mexican, and negro.

The object of the project is to determine whether Texas school children exhibit seasonal variations in their growth curves, and whether the three racial groups show characteristic or significant differences in their physical development. In order that the study may contribute to our more exact knowledge concerning physical growth processes, special attention has been given both in the preliminary plans and subsequent procedure, not only to employment of suitable equipment and good technique, but also to the collection of data under as nearly uniform conditions as possible.

This study belongs to the individualized type, inasmuch as the growth of individual children is being followed over a period of time. Individualized studies stand in contrast to the older generalized type, in which a single measurement is taken on each member of a large group. Averages are then derived from these single measurements.

The collection of data for this study has already been in progress through one and one-half school years. Some facts obtained well justify the provision for a known weight of clothing to be worn while weight is being taken, weighing at approximately the same hour of day each month, and requiring each child to empty the urinary bladder immediately before weighing. These three procedures were adopted as routine at the beginning of this study. Their importance is emphasized by an analysis of the data pertaining to them. It seems logical to present these data in such manner as will

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call attention to the performance of children as individuals. Averages that could be derived from the figures seem of less interest than the relation between actual monthly changes in nude body weight of individuals and each of the three factors to be considered in this report.

Clothing weights compared with body weight changes. Continental scales of platform type, calibrated in ounces, are used. Uniformity of clothing worn at the times of weighing is insured by the use of garments of known weight. This garment weight is checked from time to time. Thus the nude body weight of each child is obtained. Indoor clothing exclusive of shoes, wraps, pocket contents, jewelry, beads, and girls' heavy belts has been weighed at five different monthly weighing periods. Three seasons are represented: the fall weights were taken in October; the two winter, in January and February; and the two spring, in March and April.

With the assumption that a group of 100 children would constitute a fair sample, a comparison has been made between the clothing weights and the monthly weight changes of 99 white children, 51 boys and 48 girls. These children were in the fourth and fifth grades, and for the most part were 10 and 11 years old.

Four-fifths or more of the maximum monthly body weight changes for both boys and girls occurred in either a winter or a spring month, with an approximately even division between the two seasons. With two exceptions, the maximum changes were gains. More than four-fifths of the minimum body weight changes for both sexes also occurred in either a winter or a spring month. For the boys nearly twice as many minimum changes came in a spring month as in the winter; for the girls about one-third more, in the spring. Among the minimum changes the gains and losses are about equally divided, and with few exceptions are 4 oz. or less.

Maximum clothing weights ranging from 21 to 40 oz. for boys and from 9 to 31 oz. for girls, fell in the winter months in four-fifths or more of the cases. Roughly three-fifths of the minimum clothing weights varying between 15 and 30 oz. for boys came in the spring; nearly two-fifths in the fall. For the girls the minimum clothing weights, varying from 6 to 13 oz., were equally divided between spring and fall. Fewer than 10 minimum clothing weights for either sex were found in a winter month.

With the drop from maximum to minimum clothing weight in the spring, which occurred three times out of five among 51 boys and half the time for 48 girls, any gain in body weight would seem to be less than it really is, had the child been weighed in his indoor clothing. If the body weight change is a loss, then the loss is augmented by the drop from maxi-

mum to minimum clothing weight. On the other hand, a change from minimum to maximum clothing weight might conceal the fact that the child has actually lost weight, or else materially minimize the loss. The case of G. R. in Table I illustrates this possibility. His maximum clothing

TABLE I

CLOTHING WEIGHTS COMPARED WITH MONTHLY CHANGES IN NUDE BODY WEIGHT

Boys	Monthly changes in body weight			Clothing weight			Clothing weight difference equals or exceeds monthly wt. changes
	Max.	Min.	Changes greater than clothing wt. difference*	Max.	Min.	Diff.	
	lb–oz.	oz.	oz.	oz.	oz.	oz.	Times
N. C.	+1–15	−1	29, 31	29	17	12	8 out of 10
H. R.	+3–7	+1	18	35	20	15	6 out of 9
H. J.	+3–6	−2	20	32	22	10	4 out of 6
D. A.	+2–1	+1	17	31	18	13	7 out of 10
W. R.	+1–10	−3	17, 20	31	16	15	7 out of 10
F. G.	+3–13	−1	23	39	22	17	7 out of 10
H. H.	+2–13	+1	11, 17	24	17	7	6 out of 10
C. J.	+2–3	−2	12, 14, 17	31	22	9	6 out of 10
H. C.	+2–10	+1	15, 17, 19	34	21	13	6 out of 10
P. J.	+4–4	+2	12, 12, 15	36	27	9	5 out of 10
T. O.	−1–10	+3	12, 14, 18, 21	28	18	10	5 out of 10
J. J.	+2–12	+1	18, 19, 20	30	15	15	5 out of 10
G. C.	+1–15	±3	16	26	19	7	4 out of 10
C. E.	+3–1	+4	12, 15, 18	38	28	10	4 out of 10
F. J.	+2–10	−8	12, 13, 16	31	20	11	4 out of 10
E. R.	+3–1	±0	14, 15, 17, 20	34	23	11	4 out of 10
E. F.	+3–12	−6	17	29	20	9	3 out of 8
B. R.	+2–4	+4	20, 21, 21	35	17	18	3 out of 8
W. B.	+3–12	−1	18, 22	28	17	11	3 out of 10
S. R.	+1–3	±0	11, 19, 19	31	22	9	3 out of 6
N. N.	+2–4	+1	14, 15	30	22	8	2 out of 6
S. T.	+1–1	±2	17	35	29	6	2 out of 3
McM. J.	+1–1	−1	17	33	23	10	2 out of 3
G. R.	−1–3	+5	19	27	17	10	2 out of 3
D. G.	+0–14	+5	14	33	20	13	2 out of 3
B. W.	+1–15	−5	31	40	25	15	2 out of 3

* Those which were 3 or more times the difference are omitted.

weight is 27 oz.; minimum clothing weight, 17 oz.; loss in body weight from preceeding month, 19 oz. Had his body weight been increased by the 10

oz. difference in clothing weights he would appear to have lost but 9 oz. rather than 1 lb. 3 oz.

The extreme variations in clothing weights are of a distinctly higher order than the minimum nude body weight changes for most of the 99 children. By comparing each child's difference between maximum and minimum clothing weight with *all* his recorded monthly body weight changes, it was found that for approximately half the children, the difference between maximum and minimum clothing weights equalled or exceeded his monthly body weight changes, 30 per cent or more of the time. On the basis of this finding, the data for 26 of the 51 boys and 20 of the 48 girls are regarded as significant. These data are recorded in Tables I and II. Eighteen of the 26 boys or 69 per cent, and 8 of the 20 girls or 40 per cent,

TABLE II

CLOTHING WEIGHTS COMPARED WITH MONTHLY CHANGES IN NUDE BODY WEIGHT

Girls	Monthly changes in body weight			Clothing weight			Clothing weight difference equals or exceeds monthly wt. changes
	Max.	Min.	Changes greater than clothing wt. difference*	Max.	Min.	Diff.	
	lb.–oz.	oz.	oz.	oz.	oz.	oz.	Times
W. M.	+2–4	±0	12, 18	17	7	10	6 out of 9
W. B.	+1–2	±1	7	11	8	3	6 out of 10
G. A.	+2–8	+4	24, 26, 32, 40	31	11	20	6 out of 10
S. F.	+2–10	±0	10, 17	18	9	9	5 out of 10
S. M.	+2–9	±0	11, 12, 16	16	6	10	5 out of 10
C. H.	+1–3	+1	15, 15, 19, 19	18	8	10	5 out of 10
N. L.	+2–7	+3	14, 15	15	8	7	4 out of 10
J. V.	+2–4	±4	10, 15	15	7	8	4 out of 10
W. D.	+3–11	±0	13	16	7	9	4 out of 10
M. W.	+3–10	+1	22	21	7	14	4 out of 10
S. L.	+2–6	−1	16, 18, 20	20	6	14	3 out of 8
J. R.	+3–3	+11	16, 20, 21	23	8	15	3 out of 8
B. M.	+3–0	−1	14, 17, 20	17	8	9	3 out of 10
B. K.	+2–15	+4	28	20	8	12	3 out of 10
T. S.	+5–5	+1	19, 23, 26	23	7	16	3 out of 10
Y. D.	+2–10	−6	13	14	8	6	1 out of 3
S. P.	+2–7	−2	0	14	6	8	1 out of 3
R. M.	+3–2	−2	0	17	8	9	2 out of 6
W. B.	+1–2	+4	13, 17	18	8	10	2 out of 4
E. M.	+1–0	−1	16	20	5	15	3 out of 4

* Those changes which were 3 or more times the difference are omitted.

exhibit still greater significance, since one-half or more of the total number of nude body changes recorded for each child are equalled or exceeded by his clothing weight difference.

Even the values of the monthly body weight changes which fall outside the limits of the definition of significance here used, point further to the necessity of always weighing with a known amount of clothing those children whose growth is being followed month by month. Among the 26 boys there are 53 monthly weight changes which exceed clothing weight differences, but these monthly body weight changes for any child are never so much as three times his clothing weight difference. In 44 of the 53 instances the monthly body weight changes exceed the clothing weight difference by amounts varying from 1 or 2 oz. up to twice the clothing weight difference. The situation is similar for the girls. Eighteen girls have 40 monthly body weight changes which in each individual case are greater than the clothing weight difference for that girl. Out of the total 40, 35 changes exceed the clothing weight difference by 1 or 2 oz. up to twice the clothing weight difference.

This interpretation of the above data for 46 of 99 children indicates that the truth about monthly body weight changes cannot be known if the child is weighed in his varying amounts of indoor clothing.

Time-of-day weight differences compared with body weight changes. The normal fluctuation in body weight from hour to hour on any day for any individual, although recognized, is not often taken into account when changes in weight are being followed. The authors of this report, by weighing themselves at hourly intervals, each found extreme variations of 2-1/2 to 3 lbs. per day. Dr. T. Wingate Todd[1] notes that his "own weight with all precautions taken varies 5 lbs. in a day, and those of my children, 3 lbs."

In this project the weighing time is kept as nearly constant as possible. Within each half day, the variation of weighing time in successive months is seldom as much as two hours, more commonly less than one hour. Thus the time relationship of weighing hour to food intake and regularly occurring defecation is more uniformly maintained. Monthly weight changes are derived by subtracting the weights of two consecutive months taken at approximately the same hour of day, and with the usual attention to other factors affecting accuracy. Such weight changes are designated as *actual* in this report. The data for 22 children who by mistake were weighed in the morning instead of the afternoon, and the weighing repeated in the after-

[1] Personal communication.

noon of the same day, have been studied with respect to the bearing this time-of-day factor has upon accuracy in following the child's monthly change in weight. Sixteen of the children were white boys from 7 to 8 years old, the other 6, adolescent negro girls. For each of these children an *apparent* monthly weight change is the difference between his weight taken at a time other than his usual hour,and his weight at the usual hour in the preceding month, other conditions being the same.

Among 11 of the 22 children the difference between apparent and actual change was insignificant, one difference being 3 oz.; five, 2 oz.; three, 1oz.; and two, 0. The data in detail for the other half of the children whose apparent body weight change differs from the actual by 4 oz. or more are included in Table III.

TABLE III

TIME-OF-DAY WEIGHT DIFFERENCES COMPARED WITH MONTHLY CHANGES IN NUDE BODY WEIGHT

White Boys	Body weight				Monthly changes in body weight			Difference between apparent and actual change equals or exceeds monthly wt. changes
	Jan.	Change in wt. Jan.–Feb.			Max.	Min.	Actual changes greater than the difference* between apparent and actual change	
		Actual	Apparent	Diff.				
	lb.–oz.	lb.–oz.	lb.–oz.	lb.–oz.	lb.–oz.	oz.	oz.	Times
E. G.	46–4	+3–3	+2–5	−0–14	+3–3	−1	16, 18, 24	6 out of 10
S. J.	47–12	+1–4	+0–9	−0–11	+1–6	−1	18, 19, 20, 22	6 out of 10
R. A.	74–14	+2–14	+2–2	−0–12	+2–14	+5	19	1 out of 3
S. J.	47–3	+0–3	+0–10	+0–7	+1–7	+2	8, 11, 13, 15	4 out of 10
T. W.	45–13	+1–9	+1–5	−0–4	+1–9	+3	5, 6, 7, 7, 11, 11	1 out of 10
P. P.	59–5	+1–12	+1–6	−0–6	+1–12	+5	9, 10, 12	1 out of 10
Negro Girls								
H. C.	102–3	+1–4	+2–13	+1–9	+1–5	−3	0	10 out of 10
G. S.	121–8	−1–0	+1–14	+2–14	+2–10	+6	0	8 out of 10
R. V.	88–11	+3–4	+4–11	+1–7	+3–4	+6	0	5 out of 6
R. V.	91–12	+0–8	+3–9	+3–1	+3–2	+2	0	9 out of 10
M. P.	100–8	+0–14	+4–0	+3–2	+4–2	+2	52, 57	7 out of 10

* Those changes which were 3 or more times the difference are not shown.

The number of times the difference between apparent and actual change on the day of double weighing,equalled or exceeded the observed monthly

changes for each child is high, except for two boys. For one of these boys (T. W.) the body weight changes which were greater than the differences between apparent and actual change, range from 5 to 11 oz. in six changes out of ten; for the boy (P. P.) three of ten changes have values of 9 to12 oz. Since for these two children the differences between apparent and actual change are 4 and 6 oz. respectively, all of their monthly weight changes considered together would seem sufficient reason for retaining them in the significant group. The actual nude body weight changes for the boys range from plus 3 oz. to plus 3 lbs. 3 oz., and are greater than the apparent change in each case by differences of 4 to 14 oz. The total number of monthly body weight changes which are equalled or exceeded by the difference between apparent and actual body weight change, expressed in percentage, are respectively 10, 10, 30, 40, 60, and 60.

The apparent changes credit each of the negro girls with a gain, whereas one girl actually lost 1 lb., the others gained from 8 oz. to 3 lbs. 4 oz. The errors for the monthly weight changes range from plus 1 lb. 7 oz. to plus 3 lb. 2 oz. Here, the total number of monthly body weight changes which are equalled or exceeded by the difference between apparent and actual body weight change, expressed in percentage, are respectively 70, 80, 83, 90, and 100.

These data on this small group of children add to the evidence that monthly weighing should be done at some fixed hour in the day.

Urine weight compared with body weight changes. The failure of a few children to comply with the routine requirement to empty the urinary bladder immediately before weighing, necessitated reweighing them. This necessity suggested that amounts of urine commonly eliminated at one voiding be determined. Accordingly, 38 boys and 20 girls were weighed just before and again directly after voiding. This determination of urine weight was made immediately before each child's body weight was taken in April of 1930. The urine eliminated by half of the children weighed 3 oz. or less. Table IV contains the data for the other half of the group, 19 boys and 10 girls, who voided 4 oz. or more of urine, and for whom the urine weight has been compared with monthly nude body weight changes.

There is no significant difference as between the sex groups in the weight of urine passed by the individuals; the maximum figure for the boys is 16 oz., for the girls 13 oz. Neither is there a significant difference in absolute amounts of urine eliminated at one voiding by children and adults. In 11 determinations for three adult women, three weights were 8, 11, and 13 oz. respectively; eight weights fell upon 3, 4, 5, 6, and 7 oz. A consideration of

the data in Table IV suggests that a factor which may influence the body weight by an amount of 4 oz. or more must not be disregarded. Minimum

TABLE IV

URINE WEIGHTS COMPARED WITH MONTHLY CHANGES IN NUDE BODY WEIGHT

Boys	Body wt.		Wt. of urine voided	Monthly changes in body wt.			Urine wt. equals or exceeds monthly changes in body wt.
	March	Change in wt., Mar.–April		Max.	Min.	Body wt. changes greater than urine wt.*	
	lb.–oz.	lb.–oz.	oz.	lb.–oz.	oz.	oz.	Times
S. H.	49–5	−0–3	7	+1–14	2		5 out of 6
A. M.	113–1	−0–4	16	+4–2	+2	27	4 out of 7
S. L.	59–10	+1–7	4	+1–7	±2	6, 8	5 out of 10
C. W.	48–3	+0–3	8	+2–2	±3	10, 14	5 out of 10
B. L.	51–6	+1–12	8	+1–12	−2	10, 19	4 out of 8
McC. J.	70–11	+1–5	4	+2–3	±0	5, 8, 8	3 out of 8
P. G.	47–15	−0–10	6	+1–0	+4	8, 9, 10, 12	3 out of 8
P. C.	66–1	+0–9	7	+3–2	+4	9, 14	3 out of 8
P. N.	73–15	+1–3	5	+2–1	±0	8, 13, 14	4 out of 10
B. W.	91–15	+1–3	10	+2–4	+3	14, 18, 19	3 out of 10
A. B.	80–0	+3–11	7	+3–11	+3	13, 14	2 out of 10
N. C.	60–11	+1–12	4	+3–3	−4	6, 7, 9, 10	1 out of 10
R. J.	82–9	+0–9	4	+3–6	−4	6, 8	1 out of 10
W. L.	abs.		4	+4–1	−3	8, 11	1 out of 8
S. J.	76–15	+0–3	6	+3–2	+3	8	2 out of 7
R. G.	80–11	+0–10	5	+1–12	+9	9, 9, 10, 11, 14	0 out of 8
G. C.	57–12	−0–5	9	−1–7	+10	10, 11, 11, 15, 15	0 out of 7
E. H.	38–0	+1–9	4	+1–9	−4	0	1 out of 2
W. H.	63–11	+0–14	13			14	0 out of 1
Girls							
B. V.	48–10	−0–3	13	+1–2	±3	14, 18	8 out of 10
H. M.	65–3	±0–0	10	+1–6	±0	11	3 out of 5
N. A.	75–14	+0–6	9	+4–3	−4	0	4 out of 6
S. F.	78–0	+0–5	9	+2–10	±0	10, 17	5 out of 10
N. L.	60–4	+0–3	7	+2–7	+3	14, 15	4 out of 10
S. C.	46–15	+1–5	4	+1–5	±0	5, 8, 11	3 out of 7
T. R.	62–6	+1–5	5	+1–8	−4	6, 8, 13	2 out of 10
N. G.	50–5	+0–9	4	+1–11	+9	9, 11	1 out of 5
H. A.	90–13	−0–2	4	+4–0	−2	0	1 out of 5
C. P.	abs.		5	−2–4	+8	8	0 out of 4

* Those changes which were 3 or more times urine weight are omitted.

monthly nude body weight changes are frequently 4 oz. or less, and changes of 5, 6, and 7 oz. are not uncommon. For 11 of the 19 boys and 6 of the 10 girls the urine weight equalled or exceeded the monthly body weight changes in one-third or more of the total number of monthly changes recorded. For six boys, and four girls, the narrower limit of one-half or more of the total number of monthly body weight changes being equalled or exceeded by urine weight, holds true. Each of twenty-five children shares in a distribution of 60 monthly nude body weight changes greater than his urine weight. In 48 of these 60 instances, the monthly body weight changes exceed the urine weight by amounts varying from 1 or 2 oz. up to twice the urine weight. Only in 9 of the 60 instances do the monthly body weight changes approach three times the urine weight.

With respect to changes calculated from the April weighing, several individual cases are of particular interest. W. H., who had only two consecutive monthly weights, with 13 oz. of urine, practically equalled his 14 oz. gain. A. M. who eliminated 16 oz. of urine which exceeded his monthly weight change four times out of seven had two losses of 4 oz. each, one gain of 2 oz. and another of 8 oz. A. M. is one of six children whose April weight record showed a failure to gain. The actual changes of April from March body weight for these six children were respectively ± 0, -3, -3, -4, -5, -10 oz. Had the urine not been eliminated the apparent changes arranged in corresponding order would have been $+10$, $+4$, $+10$, $+12$, $+4$, -4 oz. Obviously, emptying the urinary bladder is an imperative procedure not to be neglected if the facts regarding monthly body weight changes are to be ascertained.

Summary

In following the weight of school children from month to month the data here presented show that accuracy cannot be attained unless, along with attention to the many other influencing factors, the child is weighed, 1.– in a known weight of clothing, 2.– at approximately the same hour of day each month, and 3.– with his urinary bladder empty.

FACTORS WHICH DETERMINE RENAL WEIGHT*

IX. ENDOGENOUS PROTEIN METABOLISM

By

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A CONSTANT relationship between the weight of the kidneys and the protein intake has been demonstrated (MacKay, MacKay and Addis, 1928a; MacKay and MacKay, 1930) in male albino rats. This relationship is of such a nature (MacKay, MacKay and Addis, 1928a, b) that an animal receiving no protein should theoretically have about 150 milligrams[1] of renal tissue per 100 square centimeters of body surface. An experiment has been carried out to test this possibility. Ten groups of male rats of varying age were weighed and then placed upon a diet similar to that which they had been receiving except that it was practically devoid of protein. After varying periods of time, depending upon their age—the older they were the longer the interval—these animals were killed and their kidneys weighed and other measurements made in the manner which has been described (MacKay and MacKay, 1927a). Partly because the rats disliked the diet and the caloric intake was not entirely sufficient, and partly because their body weight cannot be maintained on a protein-free diet, these animals lost weight during the period of observation. For this reason the body weight used for the calculation of the body surface[2] as a basis for comparison of the actual kidney weight with the theoretical figure was that of the commencement of feeding. The kidney weight is partly dependent on the amount of protein which a rat eats. Here we want to know

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[1] This figure and kidney weights given elsewhere refer to the average of the two kidneys. The organism actually has twice this amount of renal tissue.

[2] The use of what we term "body surface" ($11.3 \times (B.W)^{2/3}$) in this and other papers of this series as a reference standard need not be considered as having a biological significance. The constant relation of kidney weight to the "body surface" (MacKay and MacKay, 1927b) may only be a necessary geometrical relationship, but as such it serves a useful purpose. We suspect however that the relationship may be due to the possibility, that our "body surface" expression is, under normal conditions, probably a fair measure of the maintenance metabolism of the organism as a whole, and that the maintenance metabolism of the renal tissue probably contributes a constant percentage of this total and is dependent on the anatomical mass of kidney.

TABLE I

Group	Age (days)	Body weight (grams)	Body surface (sq. cm.)	Kidney weight (mgms.)	Mgms. renal weight per 100 sq. cm. body surface	Per cent deviation from theory
1	42	83	208	288	139	−7
		70	188	234	125	−17
		62	171	242	142	−5
		66	177	237	134	−11
		63	171	218	128	−15
		62	171	242	142	−5
2	58	118	263	394	150	0
		104	242	366	151	+1
		101	238	322	135	−11
		116	260	387	149	−1
		90	220	318	145	−3
		100	236	315	134	−11
3	80	185	356	500	141	−5
		195	370	582	157	+5
		230	409	666	163	+9
		170	337	544	161	+7
		170	337	545	161	+7
		184	355	561	158	+5
4	80	188	360	575	159	+6
		206	383	612	160	+6
		190	363	600	165	+10
		178	348	517	148	−1
		184	355	565	159	+5
		190	363	592	163	+9
5	122	250	434	600	138	−8
		274	461	668	145	−3
		242	426	654	153	+2
		234	416	572	137	−9
6	122	236	419	466	111	−26
		188	360	452	125	−17
		238	421	622	148	−1
		198	373	600	160	+6
		174	354	568	160	+6
		161	325	530	163	+9
7	184	326	520	906	174	+16
		304	496	728	147	−2
		330	524	804	153	+2
		332	527	828	157	+5

TABLE I (continued)

Group	Age (days)	Body weight (grams)	Body surface (sq. cm.)	Kidney weight (mgms.)	Mgms. renal weight per 100 sq. cm. body surface	Per cent deviation from theory
8	184	244	428	595	139	−7
		280	469	759	161	+7
		250	437	637	146	−2
		260	446	660	148	−1
		310	503	790	157	+5
9	274	360	556	856	154	+3
		320	513	762	148	−1
		274	461	659	143	−5
		310	503	753	150	0
10	274	358	553	880	159	+6
		346	541	871	161	+7
		358	553	853	154	+3
		350	543	773*	142	−5

* In place of using the gross body weight for computing this figure an allowance of 5 per cent has been made for intestinal contents so that the "body surface" obtained will be comparable with that on which the theoretical kidney weight is based. The "body surface" is the expression (body weight) $^{\frac{2}{3}} \times 11.3$. In this case the initial body weight was used.

how much kidney weight this rat would have when the factor of protein consumption is removed. This factor is removed and after a time, during which the effect of ingested protein may have a chance to disappear, the kidney weight is compared with that which the rat theoretically would have had at the body surface based on its initial body weight with a zero protein intake. The kidney weight actually found at death must, therefore, be referred to the same body surface for the purpose of comparison. That the arbitrary periods which were chosen for the kidneys to recede in weight, and for their weight to come into equilibrium with the zero protein intake were satisfactory, is suggested by the results.

In Table I are presented the individual observations and their relation to the theoretical figure. According to theory there should be 150 milligrams of renal tissue per 100 square centimeters of body surface if an equilibrium has been established between the weight of the kidneys and the protein intake which in this case is zero. The agreement of the figures actually found with this theoretical figure is very good, particularly when the nature of the data is considered. The greatest deviation of any one age group is

TABLE II
GROUP AVERAGES

Group no.	No. rats	Age		Body weight		Body surface	Kidney weight			Kidney wt. mgms. per 100 sq. cm. body surface	Per cent of theory	Per cent deviation
		Initial (days)	Death (days)	Initial (gm.)	Death (gm.)	Initial (sq. cm.)	Left (mgm.)	Right (mgm.)	Average (mgm.)			
1	6	35	42	66	53	179	238	242	240	135	90	−10
2	6	50	58	105	83	244	341	360	350	144	96	− 4
3	6	70	80	189	157	360	567	565	566	156	104	+ 4
4	6	70	80	189	167	360	566	587	576	159	106	+ 6
5	4	110	122	250	218	446	622	618	620	143	95	− 5
6	6	110	122	199	169	377	529	549	539	145	97	− 3
7	4	170	184	323	257	517	788	870	829	157	105	+ 5
8	5	170	184	269	227	457	676	700	688	150	100	0
9	4	260	274	316	286	509	734	766	750	146	97	− 3
10	4	260	274	351	271	546	829	857	843	154	103	+ 3

minus 10 per cent and the average deviation of the entire group of 51 rats from 150 milligrams of renal tissue per 100 square centimeters of body surface is 6.0 per cent. The average of the difference between negative and positive deviations is less than 1 per cent. The average kidney weight in relation to body surface for the whole ten groups is 149.6 as compared with the theoretical 150. The variability of the series, of which the former is an average, is 11.3 per cent which compares very favorably with the 7.0 per cent variability of the relation of renal weight to body surface in male rats of various ages (MacKay and MacKay, 1927b).

The consistent relation which has been demonstrated between the kidney weight and the exogenous protein catabolism as measured by the protein intake (MacKay and MacKay, 1931) led us to examine what relation existed between renal weight and the endogenous protein catabolism under conditions such that the effect of ingested protein was not a factor. Such conditions are those under which the experiment described above was carried out. These rats had been on a diet which is described elsewhere (MacKay and MacKay, 1927b) as the experimental male diet and which contains 18.0 per cent protein. When the observations reported here were commenced, a diet containing the ingredients listed in Table III was given. It is similar to their original diet except that it contains 2 per cent of Harris vitamin B concentrate in place of 9 per cent of yeast, the remaining

TABLE III

EXPERIMENTAL DIET

	PER CENT
Dried cooked cornstarch	68
Lard	15
Cod liver oil	9
Salt mixture (Osborne and Mendel)	4
Yeast extract (Harris Vitamine B concentrated)	2
Agar-agar	2

7 per cent, along with the casein, being replaced with cornstarch. It contained exactly 0.1 per cent nitrogen, which could not be certainly identified as of protein origin and in any case this small amount may be disregarded for the present purpose. The animals were kept in a special urine collection cage very similar to that described by Levine and Smith (1925), except that the collecting portion was entirely of glass. Twenty-four hour group urine collections were made upon all of the ten groups from the time the special diet was commenced until they were killed. At the end of each collection period their bladders were emptied by means of moderate downward pressure over the abdomen while several whiffs of ether were given

(Addis, MacKay and MacKay, 1926). The urine was collected in 10 cc. of 5 per cent sulfuric acid and the cage bottom and collecting funnel washed down at the end of each period. Total nitrogen was determined on the 24-hour specimens by the usual Kjeldahl method.

The nitrogen excretion of each group fell quickly to a minimum which then fluctuated slightly. It seems reasonable to assume that this final minimum nitrogen excretion should be a fair measure of the endogenous protein metabolism. In Table IV are given the group averages. The nitrogen figure is an average of the excretion per rat per day of the last 2 days in

TABLE IV
GROUP AVERAGES

Group No.	No. rats	Age at death (days)	No. days on diet	Body weight (gms.)	Liver weight (gms.)	Heart weight (mgms.)	Kidney weight (aver.) (mgms.)	Minimum N excretion (mgms. per rat per 24 hrs.)
1	6	42	7	53	2.31	239	240	16
2	6	58	8	83	3.37	370	350	15
3	6	80	10	157	5.38	557	566	27
4	6	80	10	167	5.81	581	576	29
5	4	122	12	218	7.14	774	620	43
6	6	122	12	169	5.37	590	539	31
7	4	184	14	257	7.77	840	829	52
8	5	184	14	227	6.79	824	688	38
9	4	274	14	286	8.57	766	750	50
10	4	274	14	271	7.18	847	843	55

groups 1 and 2, the last 3 days in 3 and 4, and the last 4 days of observation in all of the other groups. In Figure 1 these mean figures have been plotted against the mean kidney weight of each group. There is a very definite relation, almost linear, between the two. We believe that this relation is not due to any effect of the endogenous protein metabolism on renal weight but to the relation of both of these figures to the same factor, probably the maintenance metabolism of the organism.

In the experiment which has just been described, the endogenous protein metabolism and the renal weight were varied by the size of the animal which would likewise vary the maintenance metabolism and in similar degree. There is considerable evidence that the endogenous protein metabolism is determined under ordinary conditions by the maintenance metabolism. This suggests that the endogenous protein metabolism: renal weight relationship may be causal in nature. Evidence that this is not the case is presented here. Four groups of male rats 170 days of age were

given the protein-free diet we have described for 4 days. During the next 10 days, two groups continued to receive this food and one in addition daily intraperitoneal injections of thyroxin while the other two groups were given only water. After 6 more days the fourth group received neither food nor water. The nitrogen excretion of all but the first group increased. At

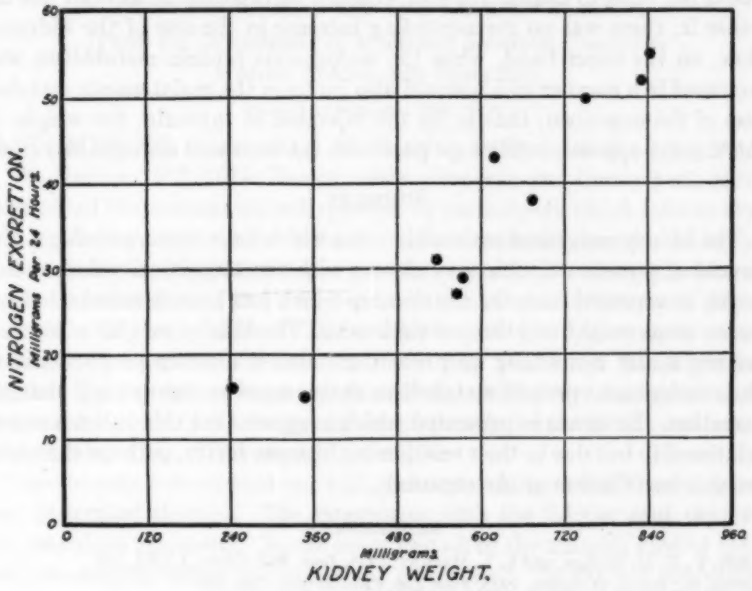

FIGURE 1.

TABLE V
GROUP AVERAGES

Group	No. rats	Body weight		Initial Body surface (sq. cm.)	Kidney weight		Terminal N. excretion	
		Initial (gm.)	Death (gm.)		Actual (mgm.)	Per sq. dcm. (mgm.)	Per rat per day (mgm.)	per sq. dcm. per day (mgm.)
Protein-free diet.....	5	269	227	457	688	150	38	8
Starvation..	4	273	170	461	603	131	83	18
Dehydration	4	278	155	465	582	125	160	34
Thyroxin...	5	253	152	437	906	208	61	14

the end of 14 days they were killed and the kidneys weighed. The results comprise Table V. Excluding the group which was given thyroxin, the rats with the higher nitrogen excretion did not have larger kidneys. In other words, when the endogenous protein metabolism was increased over that of a protein-free diet by starvation or dehydration, circumstances which would not tend to change the maintenance metabolism or at least not increase it, there was no corresponding increase in the size of the kidneys. Now, on the other hand, when the endogenous protein metabolism was increased in a manner which would also increase the maintenance metabolism of the organism, that is, by the injection of thyroxin, the weight of the kidneys approximately kept pace with the increased nitrogen excretion.

Summary

The kidney weights of male albino rats which have been receiving a diet devoid of protein coincide very closely with the theoretical values which might be expected from the relationship which has been demonstrated between renal weight and the protein intake. The kidney weights of rats receiving a diet containing no protein are almost directly proportional to their endogenous protein metabolism as measured by the urinary nitrogen excretion. Evidence is presented which suggests that this is not a causal relationship but due to their relation to the same factor, perhaps the maintenance metabolism of the organism.

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FACTORS WHICH DETERMINE RENAL WEIGHT*

X. THE EFFECT OF FEEDING DESICCATED THYROID

By

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NUMEROUS observers (R. G. Hoskins, 1910; E. R. Hoskins, 1916; Hering, 1917, 1919; Hewitt, 1920; Cameron and Carmicheal, 1920) have noted the remarkable enlargement of the kidneys which follows thyroid feeding in rats and other animals. An enormous increase in the intake of food (Schater, 1912) and hence protein is a natural accompaniment of thyroid administration and a high protein intake of itself is known (MacKay, MacKay and Addis, 1928) to lead to a marked increase in renal weight. The experiment described here was performed to determine whether the increase in renal weight which results from the ingestion of desiccated thyroid is due to the increased protein intake or to some more fundamental effect of the thyroid principle.

Experiments were carried out with diets containing 0.4, 0.8 and 1.2 per cent desiccated thyroid. The experiment with the 0.4 per cent thyroid diet was alone successful. In the other two all of the animals died of thyroid intoxication before the end of the experiment. In the one successful experiment forty male albino rats exactly 26 days of age were divided into two groups of 20 each. The care of each group was identical. The experimental details and the diet (experimental male) which they received have been described elsewhere (MacKay and MacKay, 1927a). Except for the presence of thyroid in one, the diets of the two groups were the same. The thyroid diet was prepared by intimately mixing 0.4 grams of Armour's powdered desiccated thyroid with 99.6 grams of control food. Having been placed upon the special diets when 26 days old the rats were weighed daily and their food intake determined until they were 70 days of age when they were killed and various anatomical measurements made.

In Figure 1 have been charted the growth curves of the two groups in terms of the mean daily body weight and food intake in relation to the calculated body surface during the period of experiment. Although eating

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far more than the controls, the thyroid group did not increase in weight nearly so rapidly.

The mean results of the anatomical measurements made at death are

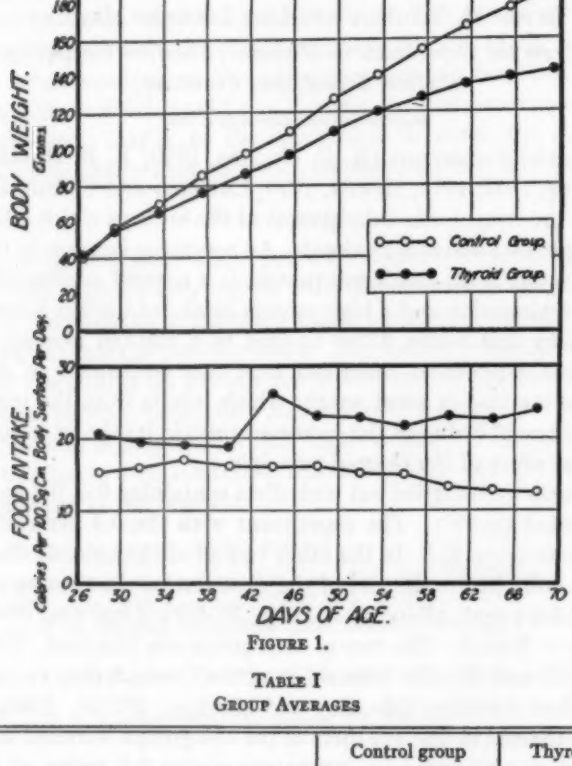

FIGURE 1.

TABLE I
GROUP AVERAGES

	Control group	Thyroid group
No. rats	20	20
Body length, mm.	196	190
Body weight (cor.), gm.	186	138
Body surface, sq. cm.	370	302
Liver weight, gm.	8.25	9.48
Heart weight, mgm.	631	1019
Kidney weight,* mgm.	701	888
Liver weight: Body surface	2.23	3.12
Heart weight: Body surface	171	338
Kidney weight*: Body surface	189	295

* Average of the two kidneys.

given in Table I. They show that thyroid feeding resulted not only in an increase in the weight of the kidneys but also of the liver and heart, an observation which has been frequently recorded before. In Figure 2 the in-

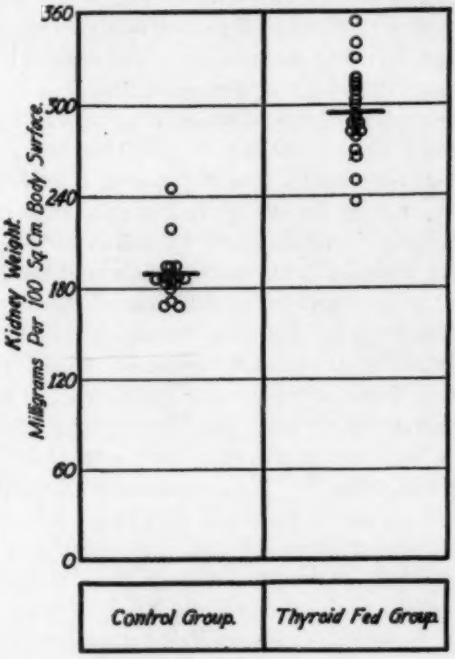

FIGURE 2.

dividual figures for renal weight are presented graphically. In Table II the observed kidney weights of the two groups are compared with their protein intakes and with the calculated weight of renal tissue for these pro-

TABLE II
GROUP AVERAGE

	*Intake—grams per 100 sq. cm. body surface per day		Milligrams kidney per 100 sq. cm. body surface	
	Food	Protein	Observed	Calculated
Control group	2.69	0.484	189	180
Thyroid group	4.93	0.887	295	198

* Average of 10-day period before death.

tein intakes, derived by means of a formula which has been shown (MacKay, MacKay and Addis, 1928) to express the relationship between protein intake and renal weight in albino rats of the age and sex and receiving a diet of the nature of that used here. It is obvious that the size of the kidneys of the thyroid rats cannot be explained solely on the basis of their increased protein intake. Some additional effect of thyroid feeding must contribute to the renal enlargement. The possibility that the increase in the maintenance metabolism of the organism may be responsible for the hypertrophied kidneys at once comes to mind. There are a number of facts which suggest that the maintenance metabolism is an important endogenous factor in determining the size of the kidneys. In normal rats of various ages the renal weight has been found (MacKay and MacKay, 1927b, c) to be directly proportional to the body surface and the latter is directly proportional to the maintenance metabolism of the organism (Lee and Clark, 1929). Then there is the present instance in which the maintenance metabolism is almost certainly greatly increased and so is the renal weight. In contrast to this circumstance are the experiments of Hammett (1927) in which he removed the thyroid gland from young albino rats.[1] From what we know of the results of this procedure in other animals and man, it would seem safe to assume that the maintenance energy demand of these rats was definitely reduced. He found that in relation to the size of the organism as a whole a marked decrease in the weight of the kidneys resulted. It is true that the protein intake of these thyroidless rats would probably be reduced and this of itself might decrease the size of their kidneys. However, recalculating Hammett's figures for his group operated when 30 days old and killed 120 days later for comparison with our data the controls have 191 mgms. and the thyroidectomized group 132 mgms. of kidney tissue per 100 sq. cm. body surface. The latter figure is considerably below that which rats receiving no protein should have theoretically (MacKay, MacKay and Addis, 1928) or are actually found to have (150 mgms.) by experiment (MacKay and Cockrill, 1931). It is planned to examine the relation of renal weight to the maintenance metabolism by direct experiment at some future time.

Summary

The administration of a diet containing 0.4 per cent desiccated thyroid to male albino rats from 26 to 70 days of age was followed by a marked in-

[1] Actually both the parathyroids and thyroids were removed for technical reasons but as Hammet (1926) has pointed out, the response may be interpreted in terms of thyroid deficiency alone.

crease in the weight of the kidneys. This increase in renal weight was greater than could be accounted for by the increase in protein intake incident to the increased food consumption which followed the administration of thyroid. This result is contrasted with the decrease in the weight of the kidneys which results from removal of the thyroid gland. It is suggested that the influence of the thyroid on kidney weight may be through its effect upon the maintenance metabolism of the organism.

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INFLUENCE OF THE STATE OF HYDRATION OF THE BODY ON THE INSENSIBLE LOSS OF WEIGHT IN CHILDREN†

By

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IN THE course of investigations on the total mineral and water exchanges in epileptic children on various regimens, it was observed that the insensible loss of body weight varied to a considerable degree with different levels of water intake and with diets which were isocaloric but of different composition. The insensible weight loss consists almost entirely of water vapor and carbon dioxide and for purposes of practical consideration is equal to the combined output of these substances minus the weight of oxygen simultaneously absorbed. As shown by the calculations of Johnston and Newburg (1), water vapor may constitute 82 per cent of the total insensible loss when the respiratory quotient is 1.00, 104 per cent when it is 0.707, and 92.8 per cent when it is 0.82. Reference to the literature reveals the fact that several investigators have observed a decrease in the cutaneous insensible water loss following restriction of the water intake.

Dennig (2) gives a small number of data from one experiment on an adult, which indicate that the total insensible perspiration is reduced during a period of thirst. His results are inconclusive, however, because his few figures are very variable. Such factors as muscular activity, room temperature and relative humidity are not considered, and the balance used for determining body weight was accurate to 100 grams only. Moog and Nauch (3), using the chamber method of Schwenkenbacher (4) for directly measuring the water evaporated from the skin of the trunk and limbs over short periods of time (7 hours), found in five adult subjects that the insensible water loss from the skin averaged from 7.0 to 40 per cent more when 3000 cc. instead of 1000 cc. of water were drunk. Schlüter (5), using the same method, found that when the fluid intake of two adults was reduced to a low level, the water eliminated through the skin fell from 22.7 to 18.7,

* Read at the annual meeting of the Eastern Society for Pediatric Research at Baltimore, May 3, 1930.

† This investigation was aided by grants from an anonymous donor and from the research fund of the Rockefeller Foundation.

and 18.3 to 15.3 grams per hour respectively. Marfan and Darlencourt (6) observed that infants with dehydration from diarrhea gave off increased proportions of water by way of the lungs. On first thought, this might indicate the possibility that the diminution in insensible water loss from the skin, as observed by Schlüter, is compensatory to an increase in the amount lost by way of the respiratory pathway during dehydration. There is no evidence, however, of a significant increase in the minute respiratory exchange in the type of dehydration resulting from simple water restriction; whereas, this is the usual effect of the acidosis accompanying dehydration from diarrhea. Erismann (7), whose technique was poorly controlled, reported finding an increase in the amount of water evaporated from the skin when the water intake was greatly increased. Peiper (8) found that the insensible cutaneous water was not increased by drinking large amounts of cold water. Laschtschenko (9), who measured the total insensible water output (skin and lungs) after increased water drinking, obtained results similar to those of Peiper.

Special interest in problems pertaining to changes in the insensible "perspiration" or insensible "loss" has been aroused recently by the observation that, under certain conditions, there is a close correlation between the energy metabolism and the net loss in body weight over a given period of time. In 1917 Soderstrom and DuBois (10), using a respiration calorimeter, found, in a fairly varied series of adult human subjects, that approximately 24 per cent of the heat produced in the body was lost by vaporization of water from the skin and lungs, when the environmental temperature ranged between 22 and 24° C. and the relative humidity between 15 and 40 per cent. Benedict and Root (11), using a highly sensitive Sauter balance (sensitive to 0.1 gm.) for determining changes in body weight over short periods of time and so-called "silk scales" (sensitive to 10 grams) for longer periods, demonstrated a definite relationship between the insensible loss and the energy metabolism in adult diabetic and hyperthyroid patients as well as in normal subjects. They supply a table by means of which the 24-hour metabolism may be approximately predicted from the hourly insensible loss measured under standard conditions. Levine and Wilson (12) have made an extensive and well-controlled study of the relationship between basal insensible loss and energy metabolism in infants and children with results comparable to those of Benedict and Root in adults. Johnston and Newburg (1) have extended the method to determine the total heat elimination over longer periods, during which various diets may be used and a limited amount of muscular activity indulged in.

The combined results of these authors have definitely established the practical utility of predicting the metabolism from measurements of the insensible loss. The only standard requirements stated so far are those having reference to the relative humidity and temperature of the environment for the avoidance of sensible sweating or chilling of the skin surfaces.

Because of the increasing importance of measurements of the insensible perspiration, and because of the indefiniteness regarding its status in different states of hydration, we have determined its value under a considerable variety of conditions in conjunction with other studies on the water metabolism in children. The results presented in the present paper are representative of a larger number of data all pointing to similar conclusions.

Plan of Study

The environmental and metabolic conditions, except for the changes induced in the state of hydration, were maintained as nearly constant as possible throughout each experiment.

The subjects, four epileptic girls ranging in age from 11 to 16 years and one boy aged 9 years, were kept in bed under sufficient bed covers to prevent chilling or sensible sweating, so far as possible. The barometric pressure, temperature, and relative humidity in the metabolism room were recorded hourly. The environmental temperature ranged between 22° C and 25° C throughout almost the entire period of observation, only occasionally rising high enough to induce slight moistness in the axilla.

The experimental day was divided into four 6-hour periods, beginning at 6:00 A.M. The method used for estimating the total metabolism was that outlined by Johnston and Newburg (1). At the beginning of each period, the patient's bladder was completely emptied, the weight was then determined on a special scale, sensitive to 5 grams and weighing to 70 kilograms, after which a standard meal of known weight and composition was given. The four meals of the day were identical in every respect and were accurately prepared in an adjoining kitchen from simple substances such as whole milk powder,[1] calcium caseinate,[2] sucrose, eggs, heavy cream, and distilled water. No sodium chloride was added, excepting in one experiment. At the end of the period the patient again voided and was weighed. The difference between the body weight at the beginning of the period plus the weight of the ingesta and the body weight at the end of the period plus the weight of the excreta, gave the total insensible loss for the period. This

[1] Klim.
[2] Casec.

value, divided by the number of hours making up the period, gave the insensible loss per hour, from which energy metabolism was predicted by reference to the tables of Benedict and Root.

Since control periods were run for each diet under normal conditions of hydration, changes in insensible loss due to differences in the respiratory quotient need not be taken into consideration. The diets for each experiment were isocaloric throughout the period of study and were calculated to furnish sufficient energy to maintain body weight under ordinary conditions.

Water balances of the body were calculated according to the method suggested by Newburgh and Johnston (13). "Total water available" (intake) includes drinking water, preformed water of the food, water from oxidation of the metabolic mixture and nonoxidation water freed by the catabolism of body tissues. The "total water lost" (output) includes water of the urine, water of the feces, water lost insensibly by way of the skin and respiratory tract and the water stored with any fat protein or carbohydrate deposited in the body.

In the course of our water balance studies varying degrees of dehydration have been produced in the different subjects by means of rigid water restriction, diuresis, catharsis, low-carbohydrate diets, or variation in the mineral content of the diet. In some instances a combination of two or more of these procedures has been employed. Superhydration has been induced by forced water drinking and frequently repeated administrations of the antidiuretic principle of the hypophysis (pitressin).

Results of Experiments

For the sake of brevity, results of the experiments are presented in a series of graphical charts and one table, which are practically self-explanatory.

The data in Chart 1 show that a significant decrease in the insensible perspiration follows the development of dehydration. The extent of the latter is indicated by the large daily water deficit and by a precipitous fall in body weight during the second half of the experiment. The control period is not entirely satisfactory because of slight sweating on the second and third experimental days. Any error, however, which might result from this during dehydration would naturally be in the direction opposite to that upon which our conclusion is dependent. It is interesting that the skin became extremely dry during the period of dehydration in spite of the fact that the mean room temperature rose on the sixth day to a point one

degree higher than that at which sensible perspiration had occurred in the control period. It is apparent from the results for the first four days that the non-ketogenic, borderline, high-protein diet had only a feeble dehy-

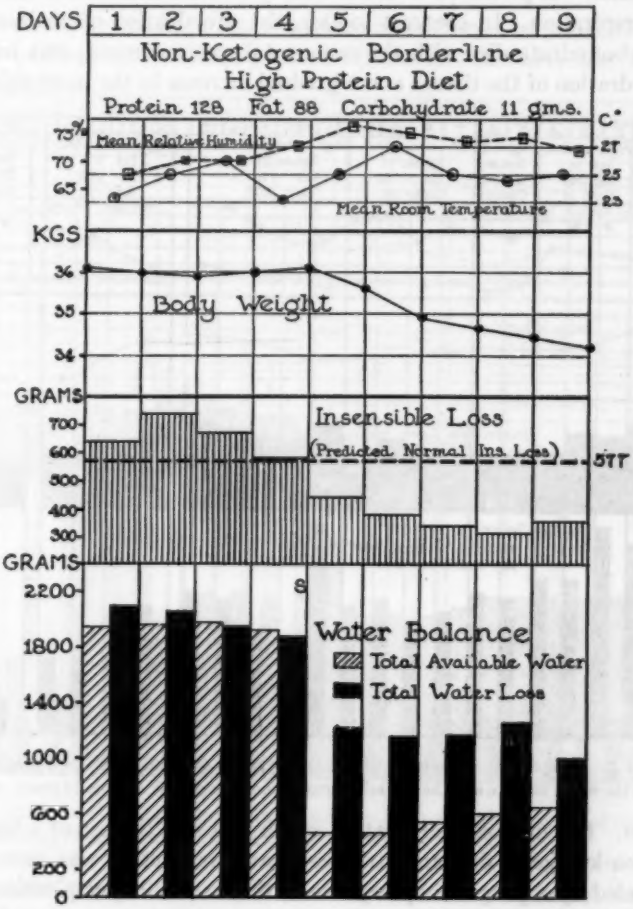

CHART 1.—Effect of marked dehydration from restriction of water intake on the insensible perspiration. "Predicted normal insensible loss" was estimated from the table of Benedict and Root according to the subject's calculated heat production. E. M., age 11 years. wt. 36 kg.

drating effect in the case of this particular patient so long as the total water intake was not restricted.

An examination of the data shown on Chart 2 reveals several interest-

ing points. The dehydrating effect of the ketogenic type of diet is clearly demonstrated by the results for the first nine days on which the diet was given. In spite of a fairly liberal water allowance, a sufficient degree of dehydration was produced to cause a fairly marked decrease in the insensible perspiration. In contrast to this, the substitution of an isocaloric, high-carbohydrate diet with the same total water content, was followed by rehydration of the tissues and a gradual increase in the insensible pers-

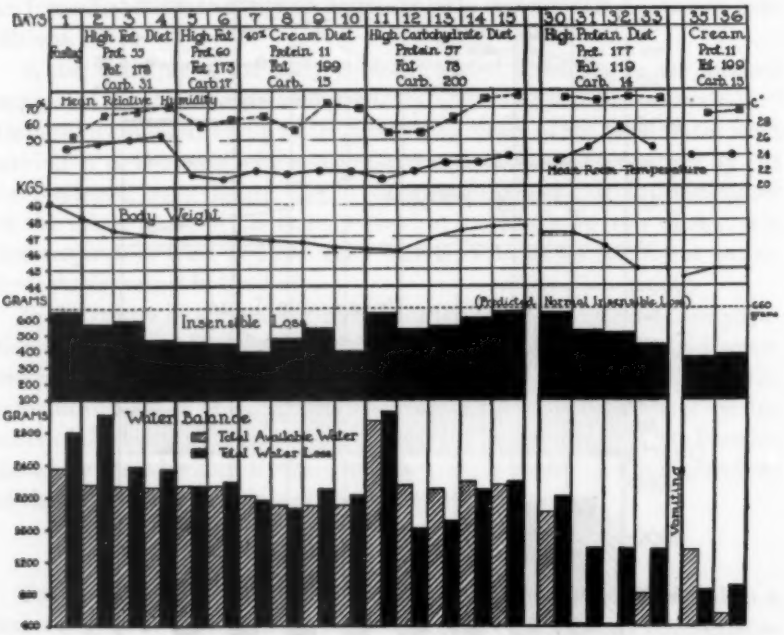

CHART 2.—Comparison of the effect of different types of diet and of different levels of water intake on the water balance and the insensible loss of body weight. W. J., age 14 years. wt. 49 kg.

piration. The marked dehydration which followed the use of a high-protein, non-ketogenic diet and stringent water restriction was likewise accompanied by a progressive decrease in the insensible perspiration. It is worthy of note that the absolute decrease was not so great as that observed during the subsequent ketogenic diet period, in which the degree of dehydration was perhaps no more intense. One possible explanation for this difference may be found in the greater specific dynamic action of the high protein diet. An increase in the total energy metabolism due to this factor would be expected to manifest itself in this way.

The relationship of the insensible perspiration to the state of hydration of the body is again shown in Chart 3. The data presented indicate that there is a rough parallelism between the degree of dehydration and the magnitude of decrease in insensible loss.

The effect of rehydration is also shown in the first and last periods of the experiment. The point of unusual interest in the first period is that relat-

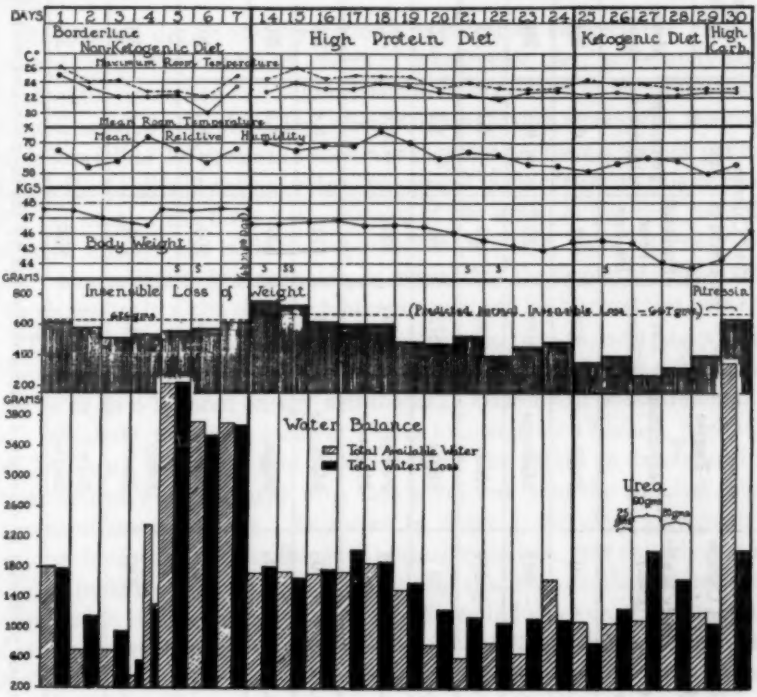

CHART 3.—Influence of different types of diet, of different levels of water intake and of diuresis on the water exchange and the insensible loss. J. R., age 16 years. wt. 48 kg.

ing to the time required for the insensible perspiration to return to its original value after completion of rehydration, as indicated by recovery of body weight. A satisfactory explanation for the observed delay when extra water alone was given is not apparent. It may indicate that the skin and subcutaneous tissues are rehydrated more slowly than the remainder of the body under these conditions in which the total sodium content of the ingesta was low. When rehydration was effected on the 30th day of the

TABLE I

Diet (1712 cal.)	Mean Room Temp. C°	Mean Relative Humidity Per cent	Body Weight KGS	Net Change in Body Water (gms.)	Degree of Dehydration	Total Calories for Period (12) hrs.			Insensible Perspiration for Period (12 hrs.)		
						Det'd (Tissot)	Predicted from Ins. loss	Percentage Difference	Measured	Predicted from Tissot	Percentage Difference
Borderline Non-Ketogenic	24	70	47.41	0	None	659	657	− 0.3	326	327	− 0.3
	22	73	46.55	− 914	Moderate	639	580	− 9.2	266	312	−14.7
	23	66	47.60	+ 259	None	630	650	+ 3.2	319	310	+ 2.9
Ketogenic	22.5	54	46.62	0	Slight	672	654	− 3.2	322	340	− 5.3
	22.5	58	45.36	−1308	Fairly Marked	666	560	−16.0	251	331	−24.1
High Protein	23.5	68	46.46	0	Moderate	693	614	−11.4	293	350	−16.3
	22	72	46.75	+ 300	Slight	694	630	− 9.0	303	350	−13.4
	22.5	61	45.42	−1233	Fairly Marked	664	500	−24.7	205	330	−38
	22.5	51	44.53	−2087	Fairly Marked	655	518	−21.0	220	324	−32
	23	50	43.90	−2752	Fairly Marked	639	508	−21.8	212	312	−32

TABLE I.—Comparison of values for heat production as determined gasometrically and by the insensible loss method under normal conditions and in dehydration. Insensible perspiration as actually measured and as predicted from gasometric metabolism measurements compared. J. R., age 16 years. wt. 48 kg.

experiment by the simultaneous administration of water and the antidiuretic hormone of the pituitary gland (pitressin) at regular intervals, recovery of the insensible perspiration from an extremely low level to an approximately normal one was very prompt. No conclusions can be drawn from a single observation of this kind, but it suggests a new angle of approach to the problem regarding extra renal regulation of water metabolism.

An extreme degree of dehydration was produced between the 25th and 29th days by the combined effects of a ketogenic diet, moderately stringent water restriction and administration in large doses of the diuretic substance, urea. On the third day, when 90 grams of urea were given, the insensible loss fell to 265 grams in 24 hours or 11 grams per hour, a level 60 per cent below the predicted normal value. In spite of the fact that on this day only 9.5 per cent of the body heat, instead of the usual 24 per cent, was dissipated through the invisible vaporization of water, there was no rise in body temperature. It is probable, however, that the degree of dehydration which results in a rise of body temperature is not far beyond this point.

The next experiment to be described was designed to ascertain whether the decrease noted in the insensible perspiration was due to dehydration *per se* or to a lowered energy metabolsim. Gasometric measurements of the metabolic rate were made at 2-hour intervals for 12 hours, beginning at 7:00 A.M. The total heat production for the period as determined by this method was compared with that predicted from the simultaneously measured insensible loss. As shown in Table I, the close agreement of values obtained under conditions of normal hydration, was entirely lacking during periods of dehydration. It is evident that dehydration of the grades observed in this experiment has no significant influence on energy metabolism and that decrease in insensible loss is relatively independent of the metabolic rate under such circumstances. The factor of respiratory quotient need not enter into the discussion here because the effect of water restriction was to reduce the insensible loss no matter what type of diet was given.

The effect of superhydration on the total insensible loss is shown by the data presented in Chart 4. In the experiment represented, the patient, a 9 year old boy, was given a basic diet of low-mineral content throughout the entire period, consisting of 200 grams of 40 per cent cream, 240 grams of cane sugar and 3600 grams of distilled water. This was divided into 4 equal parts, one fourth of each ingredient being given during the first three hours of each 6-hour period. Over certain periods, indicated on the chart

age 16 years. wt. 48 kg.

by brackets, pitressin, the antidiuretic principle from the hypophysis cerebri, or sodium chloride was administered separately or simultaneously.

Unfortunately, the insensible loss was not determined with the patient on a normal water intake. However, comparison of the values obtained for 12-hour periods on the regimen of high water intake with that estimated

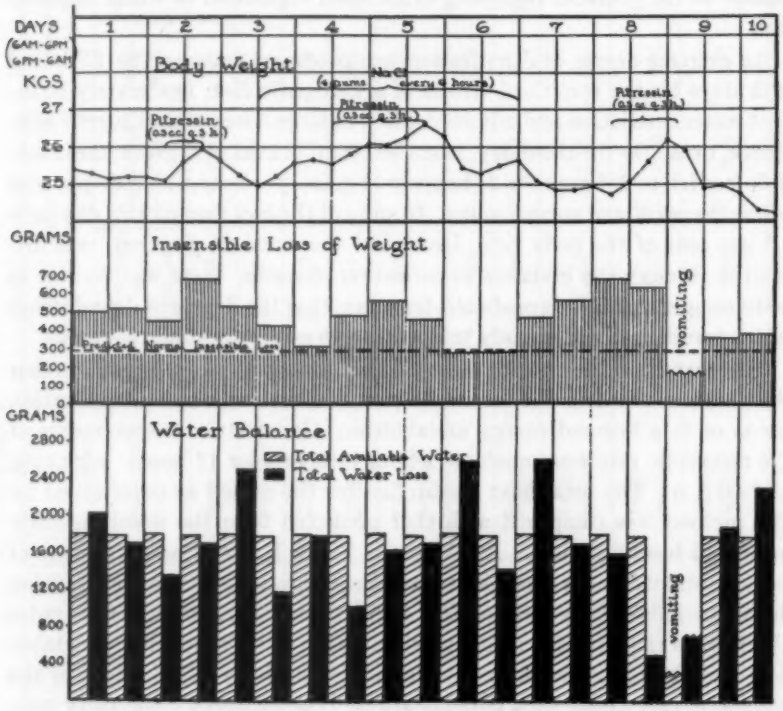

CHART 4.—Effect of "superhydration," as produced by forced water drinking and pituitary antidiureis, on insensible perspiration. Diet low in protein and minerals. Effect of added NaCl. N. F., age 9 years, wt. 26 kg.

from the table of Benedict and Root, for the same subject, shows that there was a marked increase throughout the period of study except for three of the days during which sodium chloride was being given without pitressin. With the administration of pitressin at three hour intervals, the insensible loss rose to a high peak, but again fell off on withdrawal of the extract. When 16 grams per day of sodium chloride were given, the insensible loss fell to normal on the second day, while the body weight showed an increase.

Upon resumption of the pitressin administration during the NaCl period, the body weight ascended to its highest point and the insensible loss was elevated though not to so high a level as previously. With discontinuence of the pitressin the insensible loss again returned to normal. Omission of the salt was again followed by a great increase in the insensible loss. Administration of pitressin accentuated this increase.

Discussion

The foregoing experimental data furnish proof of a definite relationship between the state of hydration of the body and insensible water loss. Dehydration, whether produced by a ketogenic diet alone, by restriction of water intake on a variety of diets, or by marked diuresis, resulted in a significant decrease in the insensible perspiration amounting in one instance to as much as 60 per cent. That the insensible loss, and not the total energy metabolism, varies under the conditions of our experiments is clearly shown. Although the CO_2 output was determined in but one case, it is practically certain that the changes in insensible loss under these conditions are due solely to changes in water output. Dehydration fever probably does not occur until the percentage of body heat dissipated by evaporation falls below 10 per cent under the conditions of our experiments. Increase in the insensible loss was consistently found to occur when large amounts of distilled water were given with a cream and sugar diet. This was still further accentuated when the antidiuretic extract (pitressin) of the hypophysis cerebri was administered in addition. Although the body weight increased when sodium chloride was given at the same time with the water, the insensible loss tended to return to the normal level. This result suggests that the amount of water lost insensibly depends to a certain extent upon the way in which it is held in the body. Further investigations should be made in this particular field.

In addition to their bearing upon the general subject of the water exchanges in the body, the results presented here have some practical importance. They demonstrate the necessity for having the tissues in a normal state of hydration ("saturated"), when the insensible-loss method is used for estimating the total energy metabolism. It is apparent also that, in addition to this requirement, extreme differences in type of diet and in mineral intake should be given consideration in the interpretation of results.

Certain of our epileptic subjects taking water *ad libitum* have been observed to develop fairly marked negative water balances and slightly nega-

tive sodium chloride balances on the ketogenic diets used in their treatment, while others, responding less well therapeutically, have not shown these effects. The insensible loss was diminished in the former cases. The reason for these differences in reaction to the diet is not clearly understood. Further investigation may show the necessity of regulating the mineral content of the diet, when metabolism determinations are to be made on persons following these unusual regimens. Under ordinary conditions, however, this precaution appears to be unnecessary, uniformly good results being obtained when the other requirements mentioned have been satisfied.

Slight alterations in the state of hydration probably do not add significantly to the errors inherent in the method of estimating metabolism from insensible loss. With a given set of conditions for any particular subject, however, the correspondence between the total insensible water loss and the state of hydration appears to be sufficiently uniform to suggest the use of net changes in body weight as an approximate measure of changes in state of hydration.

Summary and Conclusions

The total insensible loss of body weight or "perspiratio insensibilis" has been measured under standard conditons in five children ranging in age from 9 to 16 years. Variations, due to difference in diets, to restriction or forcing of water intake, to marked diuresis and to antidiuresis, have been determined.

In a normal ("saturated") state of hydration, there is a close parallelism between the insensible loss and the energy metabolsim under ordinary conditions. For practical clinical purposes, this method of estimating the energy metabolism, as proposed by Benedict and Root and amplified by Levine and Wilson and by Johnston and Newburg, offers many advantages over the ordinary gasometric methods.

Fairly marked dehydration from any cause interferes with the usefulness of the method, because the total insensible water loss is diminished to levels far below those indicated in the standard tables. Superhydration, due to excessive water drinking and to administeration of the antidiuretic principle from the hypophysis cerebri (pituitrin or pitressin), likewise interferes because in this state the values for the insensible loss may be far above those predicted from the tables. The type of diet and the mineral content of the ingesta apparently influence the insensible loss also and should be considered in the interpretation of results, when the regimen is

undergoing sudden changes or is extreme in any respect. Further investigation on this particular phase of the subject is urgently needed.

Degrees of dehydration, such as those reached in the present study, are not followed by significant alterations in the metabolic rate.**

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** The authors are indebted to Dr. L. H. Newburg for valuable suggestions regarding the technique of measuring the total water exchanges over long periods of time.

GROWTH AND REPRODUCTION ON MILK DIETS*

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ATTEMPTS to utilize cow's whole milk as the sole diet of animals have failed because of its inability to support growth and well-being. Young rats, for instance, when kept on such a diet develop a severe anemia in the course of a few weeks (1, 2). However, the addition of small amounts of the salts of iron and copper effectively prevents or cures such an anemia (3, 4). These findings have led us to study further the ability of cow's whole milk, when supplemented with copper and with both iron and copper, to support long continued growth and reproduction in rats. A study of both growth and reproduction is of course a much more exacting test of nutritive completeness and might reveal further deficiencies, if such existed.

EXPERIMENTS WITH MILK AND COPPER

In our first experiments, young rats were fed whole milk supplemented with copper alone. This was done to determine just how serious, from a nutritional standpoint, is the deficiency of iron. Three groups were taken at weaning and placed in cages with screen bottoms and fed milk supplemented with three different levels of copper, as copper sulfate. Group I, consisting of two males and four females, received 0.1 mg. Cu per 100 cc. of milk; Group II, consisting of 6 females, received 0.5 mg. Cu per 100 cc. of milk; and Group III, made up also of 6 females, received a supplement of 1.0 mg. Cu per 100 cc.

Hemoglobin determinations were made on the blood of these animals during the first few months either every week or every two weeks. Later the hemoglobin was determined at 4-week intervals.

In addition to the three groups of animals mentioned above, two other groups were available for a comparison of growth and reproductive performance on a similar diet, as part of an experiment designed to test the

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toxicity of copper added as copper sulfate. At the start of this experiment, 4 groups of four rats each were taken at weaning and fed different levels of copper. In the first group each rat received 0.25 mg. Cu daily, in the second 1.0 mg. Cu; in the third, 4.0 mg. Cu; and in the fourth, 16.0 mg. Cu. The animals of each group were kept in one large cage with a screen bottom and were fed the total copper allowance for the group in a small amount of milk in the morning. This was done in order that complete consumption would be secured. As this was consumed, more milk was added later. Hemoglobin determinations were not made on any of these animals.

The animals of the two groups receiving the highest copper dosages, namely 4 and 16 mgs. daily, did not long survive. Seven of the eight animals died in from two to four weeks. One of the rats which was receiving 4 mg. of Cu daily lived, however, for over 30 weeks, during which time it made slow but definite growth increases. It was finally discarded. These results indicate that young rats have, apparently, a large tolerance for copper, even when it is added to their diet in the form of copper sulfate. It is to be noted that the copper was offered to the rats in a rather concentrated mixture in the early part of the day and that, had it been mixed with a large quantity of milk so that it could have been consumed over a longer period, the tolerance might possibly have been higher. In other experiments in this laboratory, where a copper salt was mixed intimately with a dry ration, rats have consumed approximately 4 mg. daily over a period of 28 weeks and have grown and reproduced with every appearance of normality. It would, therefore, appear that copper is not so toxic as has been indicated often in the literature.

The two groups in this series which received the lower dosages of copper (0.25 mg. and 1.0 mg. Cu per animal daily), survived for long periods. Their performance will be discussed with the three groups described earlier. We will refer to them as Groups IV and V respectively. Group IV consisted of 2 males and two females; group V of 1 male and three females.

It is not our intention to discuss in any great detail the performance of these five groups receiving cow's whole milk supplemented only with copper, since it is known that the diet was otherwise deficient, as, for example, in iron. We shall summarize the results as briefly as possible and present typical instances in Charts 1 and 2.

It was surprising that the animals performed as well as they did. As might be expected, a marked anemia was exhibited which was of long duration in most cases. Only after many weeks, when the growth of the animal had largely ceased, did the hemoglobin approach anything like normal levels. In many instances it remained below normal throughout the

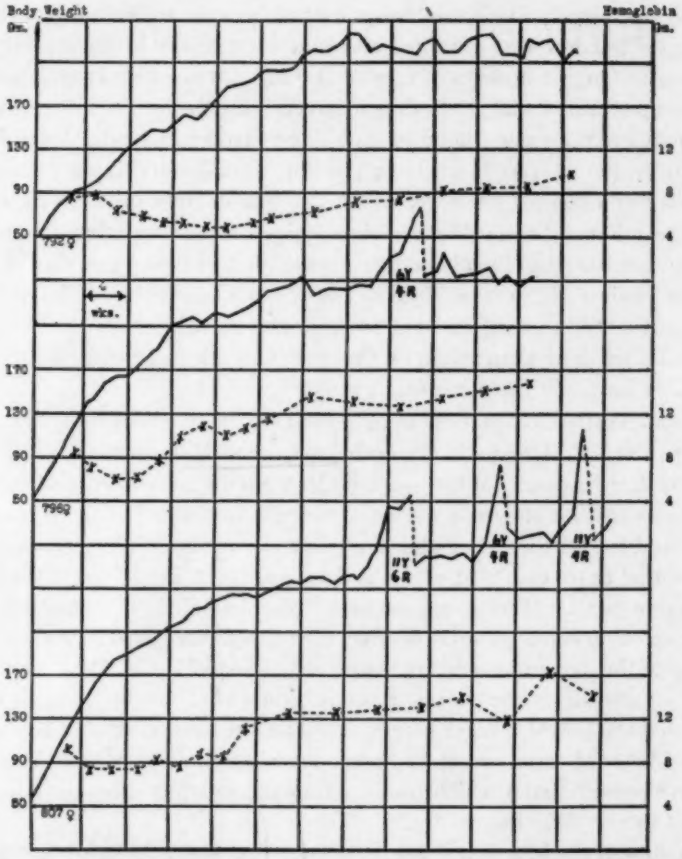

CHART 1.—PERFORMANCE OF FEMALE RATS ON A DIET OF WHOLE MILK AND COPPER.
Slow growth and long continued anemia may be noted.

No. 792 is one of the group that received 0.1 mg. Cu as copper sulfate per 100 cc. of milk. In this group there were 4 females, one of which had one litter of 3 young after over 40 weeks on this diet. The other females produced no young.

No. 796 is one of the group that received as an addition to the whole milk diet 0.5 mg. Cu as copper sulfate per 100 cc. of milk. In this group of six females only one other besides No. 796 produced young, a litter of six being born to each. No. 796 was the only one to raise her young to weaning.

No. 807 had the best record in the group of six females that received copper at the level of 1.0 mg. Cu per 100 cc. of milk. Three others produced one litter each. No. 807 raised some of each of her litters.

In this and the charts that follow the solid line represents body weight while the broken line with X's are hemoglobin values in gm. per 100 cc. of blood. The number and letter Y indicate the number of young born and the number and letter R indicate the number of young raised to weaning.

life of the animal. The growth responses of the animals were sub optimum but good weights were obtained eventually, especially in those groups receiving the higher dosages of copper (1.0 mg. Cu per 100 cc. milk and 1.0 mg. Cu per animal daily). In the group receiving the lowest copper supplement (0.1 mg. Cu per 100 cc. of milk), the two males attained weights of 290 to 300 grams after 28 weeks on the diet. Females in this same group (in all but one case) attained weights of 240 gm. or over in 26 to 28 weeks. However, hemoglobin values remained quite low, never getting above 10 or 11 gm. per 100 cc. of blood and being generally less than 7 gm. during most of the lifetime of the rats. Four of the animals succumbed to infection of the respiratory tract after 40 or more weeks, and the other two were discarded after more than a year on the diet. Growth and well being were invariably better in the other four groups.

Opportunities for mating were provided for all the females in these groups either with males in the same group or with stock males which were placed in their cages. When stock males were used these were changed at intervals so that they did not have to exist for extended periods on the milk and copper diet. Females in advanced pregnancy were segregated in individual cages provided with shavings as litter. They were returned to their groups after their young were weaned or had died. Living young were born in all groups. A total of seventeen litters was secured from the females of the five groups during the period of 47 to 55 weeks that they were kept on this diet. The poorest reproduction was shown by Group I which received the lowest level of copper. Among the four females of this group only 1 litter of three young was born. In all groups except one there were always some females which produced no young while other females produced two or three litters.

In view of the fact that it has been suggested that milk is low in vitamin E, we were on the watch for any evidence of resorption of young but found no indication of it in any instance. To further test this point we made daily vaginal smears on two females of Group IV that, although well grown and apparently vigorous, had not produced young as had the other female in this group. For a period of about 160 days vaginal smears were made daily to detect ovulation and any mating. During that time ovulation was very irregular, one female having a dioestrum period at one time of 85 days while the other went for 66 days without ovulating. No evidence of mating was ever found even at such times as they did ovulate, although the male in the group was known to be fertile. It seems, therefore, that the lack of fertility in some of the females on this diet must be ascribed to some other

disturbance than that of resorption of fetuses. This point will be discussed in subsequent pages.

The mammary function of females on this diet of whole milk and copper was also poor. Many of the litters born were not suckled, and they died in the course of a few days. Some individuals from nine of the litters were, however, suckled and raised and were eventually weaned and placed on the same diet that their dams had received. Representatives of all the groups except Group I were present in the second generation. The poor mammary function of the dam was further shown in the slow growth of these young during the suckling period. When weaned, between three and four weeks of age, they weighed generally from 38 to 44 grams which is 10 to 12 gm. below the weight of young of the same age from our stock colony. It was noted also that for some time before they were weaned they were getting most of their nourishment from the dam's feed dish and not by suckling.

It must, however, be emphasized at this point that nothing in the way of paralysis, or screaming or running fits was ever noted in any of the young from these milk fed animals. These symptoms have been observed by others (5, 6) in young suckling rats and have been explained as being caused by the specific lack of vitamins E and B. We observed no such symptoms.

The second generation consisted of 20 females and 20 males divided between 9 litters. Some of the earlier of these litters were maintained for periods of 51 to 52 weeks on the identical diets that their dams had received. Other of the later litters were discarded after about 40 weeks. Opportunities for mating were provided as all litters contained individuals of both sexes.

The growth and reproduction of the second generation was practically as we have described above for the first generation—hence no additional discussion is necessary. A total of 7 litters of young, most of which were alive at birth, was born. Of these, 4 litters were nursed more or less successfully, so that altogether 12 individuals were weaned and placed on identical diets, representing the third generation.

In Chart 2 are presented the records of two of these third generation animals (No. 1499 ♀ and No. 1454 ♂). Long records are not available on any of the individuals, of this generation. As may be seen, however, initial growth is fair in spite of the marked anemia. Vaginal smears were made on the females and the ovulations of No. 1499 are indicated on the chart. These are at a rate much below normal. It may be noted that the male, No. 1454, was shown to produce viable sperm since he sired two small litters of young, none of which, however, lived.

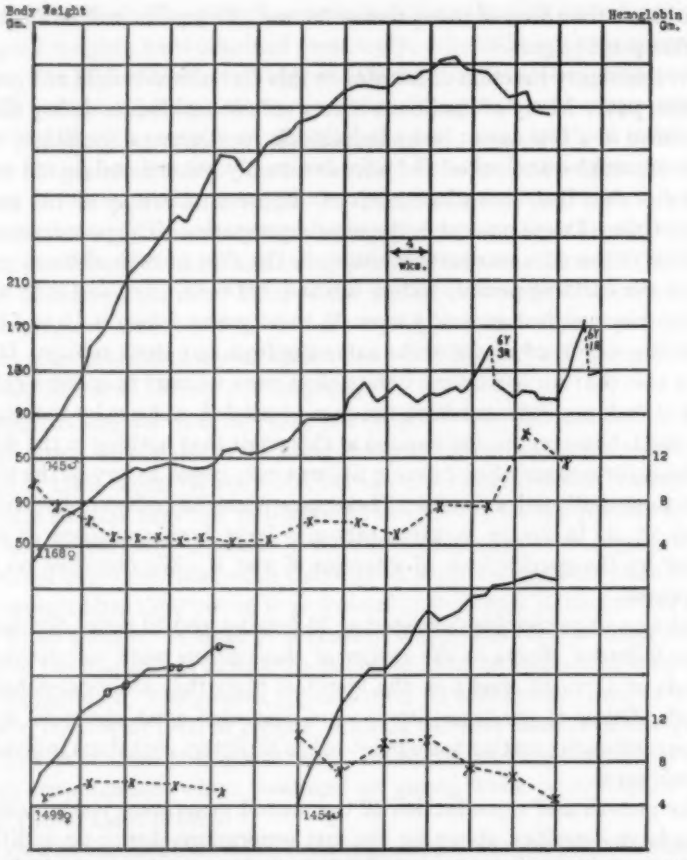

CHART 2.—GROWTH AND REPRODUCTIVE PERFORMANCE ON A DIET OF WHOLE MILK AND COPPER.

No. 945 received 1.0 mg. Cu daily in addition to his diet of cow's whole milk. Growth as may be noted was good. He was one of a group consisting of three females besides himself. One of the females produced three litters of 9, 9, and 5 living young, of which he was the sire. The last litter was born one week before he was discontinued. He attained to a greater weight than other males on similar diets.

No. 1168 was one of a group of five second generation animals (2 ♂ and 3 ♀). They received 0.25 mg. Cu daily per animal in addition to milk diet. One of the ♂'s died after 33 weeks and one of the ♀s after 38 weeks from pulmonary infection. The other female, besides No. 1168, produced one litter of 6 young of which she weaned 3. As instanced by 1168, growth was poor and anemia was marked.

Nos. 1499 and 1454 are two representatives of third generation groups on milk and copper. No. 1454 was known to be the sire of two small litters of living young.

In this and the following charts the O's on the growth curve indicate ovulations.

Experiments on Milk Supplemented with Copper and Iron

During the past year we have carried on experiments (in addition to those described above) in which the milk diet was supplemented not only with copper but also with iron. In view of our experience with the performance of animals on the copper supplemented milk, it was assumed that the further addition of iron might improve the diet so that better growth and better reproduction would result. It was also thought that, should the iron and copper supplemented milk diet still prove deficient, the possibilities of discovering the nature of these deficiencies would be greater when both the inorganic elements, *viz.*, iron and copper, were present in sufficient amounts.

The diet used was cow's whole milk supplemented with 0.5 mg. Cu as copper sulfate and 1.0 mg. Fe as ferric chloride per 100 cc. of milk. In one group a slight variation was made in that the iron was fed at the rate of 0.5 mg. Fe per animal daily in a small amount of milk and as this was consumed more milk was added containing the above copper concentration. The animals were placed on the milk diet at weaning and kept in cages supplied with screen bottoms.

Both males and females were started on these diets either in groups by themselves or in groups where litter-mate brothers and sisters were present. Because of a marked testicular degeneration noted in males confined to this diet, a discussion of their performance will be set forth in another publication. The present discussion will be confined to the performance of the females.

A total of 19 females (first generation) on the milk, copper, and iron diet was studied. We present typical instances of their performance in Chart 3 in which are shown growth, hemoglobin values and reproductive performance. In brief, it may be said that the growth was of the same order as that secured in the previous experiments in which copper only was added to milk. Good weights were eventually secured, but neither the increases in weight nor the final weights attained were optimum. The hemoglobin values remained fairly uniform throughout and no marked anemia was present, but the values remained close to 12 gm. per 100 cc., which is probably close to the lower limits of normal values. Probably due to the absence of the anemia, less evidence of infections of the respiratory tract and a slightly better condition in general was observed in these females as compared with those in the earlier experiments on milk and copper.

Among the first animals to be started on this diet were 6 females which, after they were well grown, were given the opportunity to mate with males

on a similar diet. Following a lengthy period in which there was no evidence of mating or pregnancy, they were mated with stock males and daily vaginal smears were made on them in order to gain more insight into their reproductive behavior. The taking of vaginal smears was started about 20 weeks after they had been on the diet and was continued until they were discarded. Three of the females were carried along on the diet for a total period of 56 weeks; two, because of very poor ovulation, with absence of mating, and rather poor general condition, were discarded after 45 weeks; and the sixth one died after 27 weeks on the diet. In females that mated and became pregnant, vaginal smears were discontinued after the implantation sign (R.B.C.) was observed. Just before parturition, the females were segregated individually in cages provided with shavings as litter and allowed to bear their young and suckle them if they could do so. When the young were either all dead or else weaned, the females were returned to the group and daily vaginal smears made again.

Of the above six females, three mated and bore young. One had three litters of 8, 9, and 6 young but raised only 6 of the first litter. The second had one litter of 6 which she did not nurse, while the third had a total of five litters of which she successfully raised three. This last individual is No. 1262 of Chart 3. Poor mammary function was evidenced here again in the lack of ability to nurse the young and in the slow growth of the young that were raised. The young rats were considerably below the weight of young from our stock colony at the regular weaning age of 3 to 4 weeks.

The litters which were raised were continued on the same diet. The majority of these animals were, however, males and very little data are available on the performance of second generation females. One such female produced one litter of 7 young which she did not raise. She had been on the milk, iron and copper diet for about 22 weeks when her litter was born. Growth in the second generation was not so good as in the first.

Other females of first generation groups, started later than the one discussed above, had similar histories. Not all of the 19 females (first generation) kept on this diet had the opportunity to mate, because of the fact that we wished to study more thoroughly the ovulatory rhythm without the disturbance of mating. In the last group started on this diet, a group of 7 females, observations were made to determine the time at which the vaginal orifice opened. As soon as the orifice opened, daily vaginal smears were made. In one of these females the vaginal orifice opened after 7 weeks on this diet (age 78 days), in another it opened after 9 weeks (age 91 days), in two others it opened between 10 and 11 weeks after being started on the

diet, in the fifth animal it did not open until after 15 weeks on the diet, while in the remaining two animals the vaginal orifices have not opened to date (22 weeks on the diet—age around 175 days). Long and Evans (7) have shown that the vaginal orifice is established in normal females at an average age of 76.5 days.

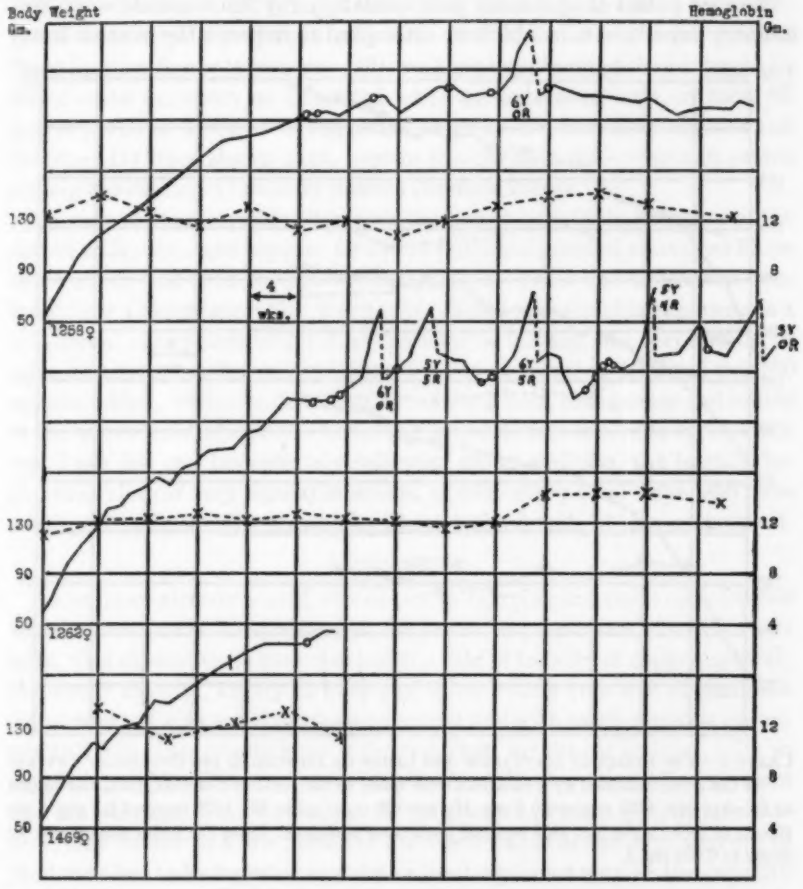

CHART 3.—PERFORMANCE OF FEMALE RATS ON A DIET OF WHOLE MILK, IRON, AND COPPER.

Nos. 1258 and 1262 are two of a group of six females, three of which did not reproduce. The performance of No. 1262 is the best we have encountered on milk diets. The | on the curve of No. 1469 indicates the time at which the vaginal orifice opened. Ovulations were slow and irregular on this diet.

In those animals in which the vagina opened naturally, ovulation has been very poor as is shown in Chart 3 (Rat No. 1469). This poor ovulation has been noted in all females on this diet on which vaginal smears were made. Even in the case of No. 1262, which made such an exceptional record, the early ovulations were at a very slow rate.

Because of this exceptionally poor ovulatory rhythm we made some preliminary experiments in which we attempted to improve the ovarian func-

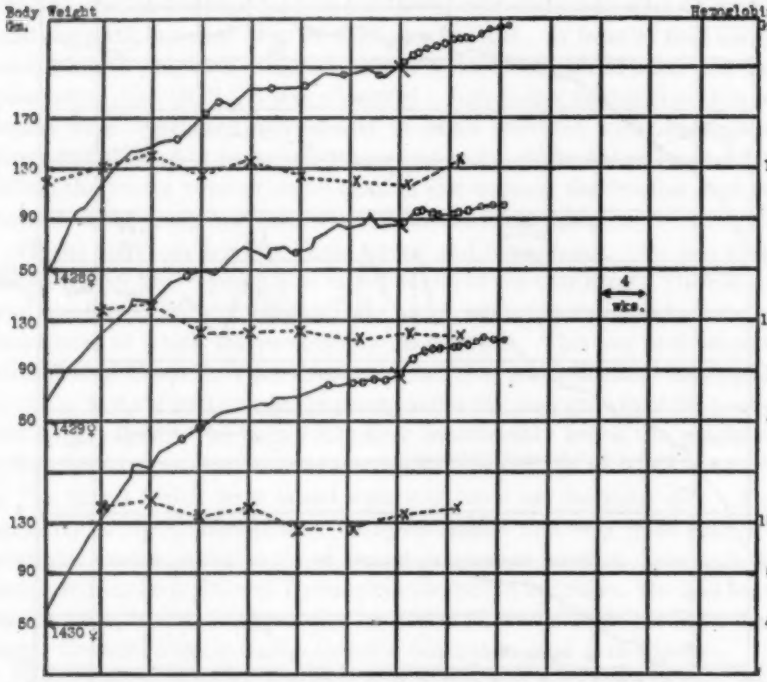

CHART 4.—THE EFFECT OF MANGANESE AND IODINE ON INCREASING THE OVULATORY RHYTHM.
At the point indicated by \ additions were made to the basal diet of milk, iron, and copper as follows: No. 1428 received 0.5 mg. Mn per 100 cc. of milk; No. 1429 received 0.2 mg. I per 100 cc. of milk; while No. 1430 received both these additions. Later the iodine dosage was reduced to 0.002 mg. I.

tion by the addition of small amounts of inorganic substances. The known effect of low vitamin B intake on suppressing ovulations (8) and the slow growth of the young suggested that the milk diet might be low in vitamin B. On the other hand, it did not seem to us, in view of the continued production of young in several generations with the absence of any specific dis-

turbance in them during the suckling stage, that vitamin B could be low. Consequently we attempted to improve the diet by the addition of inorganic substances only.

The two elements which first occurred to us as possibly being present in insufficient amounts were manganese and iodine. Manganese has been found by Titus *et al* (9) to supplement iron effectively in curing the nutritional anemia produced by a milk diet and, while we could not confirm these findings in our laboratory (10), we have kept in mind that it might be found to be necessary or beneficial when animals were kept on milk for longer periods. Iodine was suggested to us by the fact that Maurer and Ducrue (11) have shown that, next to the thyroid, the ovary and uterus among the organs of the body possess the most iodine.

In view of these considerations we decided to add these elements to the diet of milk, iron, and copper. Of the 19 females discussed above, we chose three which had been on this diet for about 28 weeks and which had ovulated only at long intervals. To the diet of one was added manganese as a solution of manganese chloride at the level of 0.5 mg. Mn. per 100 cc. of milk, to another iodine as a solution of KI at the level of 0.2 mg. I per 100 cc. was added, while the third female received both manganese and iodine in the above concentrations. Later the iodine dosage was reduced to 0.002 mg. I per 100 cc. Immediately following either addition, the females began to ovulate at very regular intervals, namely every 4 to 5 days, and have continued to do so. We present in Chart 4 the details of this experiment.

Discussion

As we have already stated, our object in carrying out these experiments was to obtain more information on the nutritive properities of cow's whole milk. Our observations have covered the field of toxicity of copper, growth, ovulatory rhythm, ability to bear and nurse young on a sole diet of milk supplemented with small amounts of copper and with small amounts of copper and iron. Our results have indicated, that, for the rat at least, milk so supplemented is still not a perfect food. Growth, as we have shown, is not at a normal rate. This we assume, in the absence of more specific information, may be due to a low intake of calories because of the large amount of fluid that has to be ingested and that a limiting factor may be the capacity of the animal to handle sufficient quantities. In preliminary experiments in this laboratory we have doubled the caloric content of the milk by adding, among other things, whole milk powder, and have observed much better rates of growth in the early weeks. Whether this is entirely due to an increase of calories remains to be seen.

The poverty of milk in certain specific vitamins, notably vitamin B, has been the subject of much discussion. Not wishing to go into a complete discussion of the literature, we would refer to a recent article by Daniels, Jordon and Hutton (12) in which they deal with this subject and its relation to the growth of suckling young in milk fed rats. We prefer to explain the slow growth of young born to our milk fed females as being due to poor mammary function in the mother (quantitative) and not to any lack of known vitamins in the mother's diet. We are influenced in this opinion by the total absence of any specific symptom in the young, such as muscle incoördination, screaming or running fits, or collapse, and the fact that young, showing evidence of being nursed at all, grew and were weaned even though the growth rate was slow. It is noteworthy that certain females (see 1262) were able to produce several litters of young and nurse them more or less successfully on this diet without themselves or the young showing distinct evidence of lack of vitamin B.

The decreased lactating ability of the females we are unable to explain unless it is that the low caloric intake does not allow full mammary development. It is altogether possible that the decreased mammary function is another expression of deranged ovarian activity, as are the irregular and slow ovulations observed on these milk diets.

The taking of daily vaginal smears on all of the later animals has given us a better insight into the reasons for the poor reproduction of these milk-fed females. It is apparent that sexual maturity comes much later on this diet than in normal animals. Ovulations, even when once initiated, come generally at very long and irregular intervals and mating is not always accepted during these ovulation periods. The exact nutritive deficiency that is responsible for this condition we cannot yet decide, but the results so far secured with the addition of small amounts of manganese and iodine suggest that these inorganic elements may play an important part in the normal acitivity of the ovary and that they are not present in sufficient concentration in the milk which we used. It is of interest in this connection that Daniels and Hutton (13) secured better reproduction in milk fed animals by adding certain inorganic materials which included manganese and iodine.

Our study of the reproductive performance of milk-fed females has shown that there is no evidence of lack of vitamin E in animals subsisting on the whole milk used by us. This is of importance in view of the fact that milk has been thought to be low in this factor. In no instance have we found any evidence of resorption of young in pregnant females. Our experience has been that in those females in which mating and pregnancy had

been established by vaginal smears, young were always delivered. This is of further interest when it is considered that a marked testicular degeneration has been noted in males confined to a diet of milk, copper, and iron. These findings will be reported in another publication.

Summary

A study has been made of the growth and reproductive performance of rats on diets of cow's whole milk supplemented with copper and with copper and iron.

On a diet of whole milk and copper a chronic anemia, due to the low iron intake, was observed. Growth and reproduction were below normal, although fair weights were eventually reached and living young were produced. Many of the young born were not nursed, and those that were suckled grew more slowly than young from our stock colony. Third generation animals were secured.

On a diet of whole milk, iron, and copper, no anemia was observed but growth and reproduction were still sub-normal. The rearing of young was poor, due to poor mammary secretion. It is suggested that subnormal growth and poor rearing of young may be due to the low caloric diet. No specific evidence of low vitamin B was observed.

By means of daily vaginal smears it was established that subnormal reproduction was due to late sexual maturity and very poor ovulation. Some females ovulated at very long intervals and never mated. Preliminary experiments indicate that the addition of small amounts of either manganese or iodine, or both, greatly improves the ovulation rhythm in females on the milk, copper, and iron diet.

No evidence of lack of vitamin E was noted in females on these milk diets.

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MALE STERILITY ON MILK DIETS*

By

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IN THE immediately preceding paper we have discussed the effect of supplementing a diet consisting of cow's whole milk with copper and iron salts on growth and reproduction in the female rat. In this paper we wish to report the occurrence of male sterility on the same diets. We first noted testicular degeneration in two male rats on this diet in some of our early experiments when studying the supplementary effect of copper and iron on the cure and prevention of anemia caused by milk diets. In those experiments it was our custom to take young rats at weaning and put them on a sole diet of milk until a marked anemia had developed. When the hemoglobin level was sufficiently reduced, the rats were taken for experiments in which corrective measures were tried. Out of each of two groups of such animals that had received an addition of iron and copper to the basal diet, a male and a female were retained to determine if continued growth would take place and if they would reproduce.

The history of these four animals need not be detailed here, but it was eventually found that both males were sterile while one of the females mated with a stock male produced living young on this diet. One of the males died after having been on the milk diet for 39 weeks while the other male was continued for more than a year. In both cases evidence of sterility was noted as indicated by infertile mating and by the presence of non viable sperm in the vaginal plug. At death the right testis from each was saved for histological examination.

The testes were small and extremely edematous, and smears from the cut epididymis showed complete absence of sperm. Later histological sections of the testes showed complete degeneration as evidenced by the absence of germinal epithelium. Because of the fact that these animals had lived

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through an early period of severe anemia and because they had been on such low levels of copper intake that their hemoglobin levels remained subnormal, it was thought that the long continued anemic condition might have been responsible for the testicular degeneration. It was also thought, at that time, that the sterility might have been occasioned by the low vitamin E of the diet since many workers have brought forward evidence that milk is low in vitamin E. In later pages we will discuss our findings in this regard since we do not believe this to be the cause of the sterility observed in our experimental male rats.

In later experiments in which both male and female rats were made to subsist for long periods on whole milk supplemented with iron and copper, we again observed sterility in the males, characterized as before by the complete disappearance of the germinal epithelium. In nearly all of these experiments, iron was added in the form of a solution of ferric chloride at the rate of 1.0 mg. Fe per 100 cc. of whole milk, and copper in addition as a solution of copper sulfate at a level of 0.5 mg. Cu per 100 cc. of milk. These additions were made to the diet from the time that the animals were weaned and hence their hemoglobin level was maintained at approximately a normal figure. It would seem, therefore, that anemia, acute or chronic, was not an essential factor in the cause of this sterility. All of the first generation males on this diet grew well, reaching weights around 300 gm. after 16 weeks.

In the first of the later experiments a group of 4 males was kept on the above ration for 15 weeks after which the males were caged individually with females on the same diet. After some weeks, when no pregnancies resulted, daily vaginal smears were started on the females to determine if mating was taking place. A few days later a vaginal plug was found in one of these females but very few sperm could be found and those seen showed signs of abnormality as none were motile. The female did not become pregnant. Following this observation, it was decided to subject the males to test matings with young normal females on our stock diet. The normal females were subjected to daily vaginal smears and when any was found to be in the beginning stage of ovulation she was placed in the cage of one of the males. The following day search was made for a vaginal plug or residual sperm and the female was returned to her own cage. Test matings were secured on three of the males, but the fourth one never copulated although several opportunities were afforded. From these matings, pregnancy never resulted and spermatozoa were either abnormal in appearance, or absent from the vaginal contents.

The first infertile mating observed occurred when the males had been on the diet about 21 weeks at which time the male in question was 175 days old. In order to allow the degeneration to proceed to the maximum, the males were continued on the diet for several weeks longer. Two were killed after 35 weeks and the other two after 38 weeks. The right testis was fixed and saved for histological study while the other was used for determining the fresh weight and for making smears from the left epididymis. These animals are numbers 1263, 1264, 1265, and 1266 of Table I.

In a second group started on this diet three males were present. These were allowed to continue for 32 weeks after which they were killed; no mating history was secured on them. They are numbers 1325, 1326, and 1329 of Table I.

When it was apparent from the mating history of the first group that degeneration was to be expected, a slight modification was made in the diet of a later group. This group of six animals, littermates, made up of three males and three females, received iron at the rate of 0.5 mg. Fe each daily in a small amount of milk in the morning and after this was consumed they received more milk containing the same copper concentration as described above. This was done because it was the iron that was suspected of causing, either directly or indirectly, the injury to the testis. Therefore, by feeding it all in a small amount of milk, the major part of the diet was not subjected to its prolonged influence. The group was kept in one large cage supplied with a screen bottom.

Approximately 10 weeks after the animals were started, vaginal smears were made daily on the females. Two days later the remains of a vaginal plug were found in one of the females. This showed very few sperm. The sperm were abnormal, having swollen heads and being tangled in masses. No free sperm were found in the deeper portions of the vaginal canal, which would indicate that none of the sperm were motile. Twelve days later the same female mated again, and again abnormal sperm were found in the vaginal contents. The female did not become pregnant and ovulated 10 days later. This indicated that even as early as 10 weeks on this diet marked degeneration was present in the testes of these males. Immediately following this one of the males (No. 1425) was killed and his right testis fixed for histological study. The other two were separated from the females for further testing with young, normal females.

Two such test matings were secured during the next few weeks with two young females from our stock colony. In both cases the sperm found in the vaginal canal gave evidence of abnormality, and in no case did either fe-

TABLE I
TESTICULAR DEGENERATION ON DIETS OF MILK, IRON, AND COPPER

Rat no.	Initial weight	Final weight	Weeks on diet	Weight of testis (trimmed)	Condition of testis	Epididymal contents	Histological findings
	(gm.)	(gm.)		(gm.)			
1263	55	340	35		Very edematous	No sperm seen.	Germinal epithelium completely absent, tubules reduced in size.
1264	58	320	35	0.88	Very edematous.	Very few, very long, non-motile, D sperm, epoorly defined hads.	Same as 1263—complete degeneration.
1265	58	326	38	0.80		Very few sperm-like structures—unusually long with no heads — two fused together.	Tubules much reduced in size and also shrunken. No germinal epithelium.
1266	58	274	38	0.79		No sperm seen.	Same as 1265—no germinal epithelium.
1325	61	337	32		Edematous.	Few sperm. Non-motile—some headless, some had swollen heads.	Most of tubules denuded of germ. epithelium. Some few tubules possess spermatagonia. One or two tubules possess giant cells.

1326	58	353	32	0.81	Edematous.	Same as 1325.	Complete absence of germinal epithelium. Tubules small and misshapen.
1329	59	370	32			More sperm than 1325—non-motile, heads broken off, swollen heads.	Complete absence of germinal epithelium, same as 1326.
*1425	58	230	12	0.60	No marked edema	Some sperm—*one* seen moving in whole smear—others abnormal.	Germinal epithelium gone in all but few tubules. In these are giant cells.
*1427	58	185†	16			Large number of sperm—some motile, majority of sperm are clumped —"tufts of hay."	Many outside tubules show complete degeneration. Other in less advanced stage have giant cells. Some tubules in center are normal.
*1426	60	276	24	1.46		Many motile sperm apparently normal.	All tubules but 3 or 4 are apparently normal. These 3 or 4 show complete or almost complete absence of germinal epithelium.

* These three animals received iron dosage at rate of 0.5 mg. Fe daily in small amount of milk in morning and copper supplemented milk later. The other animals in this table received iron at rate of 1.0 mg. Fe per 100 cc. of milk.

† This rat weighed 233 gm. the week before he was killed. The drop in weight was occasioned by an infection in left nostril with swelling causing his nose to curve to right so that his incisors did not occlude. He ate very poorly during the last week.

TABLE I (continued)

TESTICULAR DEGENERATION ON DIETS OF MILK, IRON, AND COPPER

Rat no.	Initial weight	Final weight	Weeks on diet	Weight of testss (trimmed) (gm.)	Condition of testis	Fpididymal contents	Histological findings
1448	59	203	15	1.16	No marked edema.	Very many motile sperm.	Normal testis.
1447	51	190	18	0.53	Edema.	Few sperm but none normal and no motility.	Germinal epithelium disappearing from some tubules. In central tubules epithelium not differentiated. Epithelium deep but no sperm apparently produced.
1449	52	198	23	0.58	Very edematous.	No sperm.	Only 4 or 5 tubules show any germinal epithelium. Rest show complete absence of epithelium. All tubules small.
1446	50	205	25	0.8	Very edematous.	No sperm.	Absence of germinal epithelium, much edema, tubules small.
1450	52	192	25	0.55	Very edematous.	Few degenerate sperm. No motility.	75 per cent of tubules show almost complete absence of germinal epithelium. Remainder have more or less germinal epithelium—some having deep epithelium but little differentiation.

male become pregnant to these matings. It is to be noted that both males were in the same cage and it was assumed that both had participated in the copulations. Some time later one of the males (No. 1427) was killed because of an infection in his left nostril which interfered very much with his eating, and his right testis saved for histological sectioning. The remaining male (No. 1426) was continued on the same diet.

He was further tested about 4 weeks later with normal females and found to be apparently normal. The sperm seemed of normal morphology and females became pregnant. This was rather surprising but further tests proved that he was able to produce normal sperm because four litters of living young were sired by him. He was killed after 24 weeks on the diet and a description of the histology of his testis is reported in Table I.

The above described ten animals are all of the first generation male rats that have been so far examined on the iron and copper supplemented milk diets. We have in addition, however, had the opportunity to study a litter of second generation animals, produced on this same diet (1.0 mg. Fe and 0.5 mg. Cu per 100 cc. milk), by a female that was mated with normal males. It so happened that of the six raised by her, five were males. It was thought that the condition would be much more severe in second generation males, but our observations being limited to these five, are not sufficiently extensive to determine this point. In Table I we have presented the findings which were secured by killing the second generation individuals at varying dates (Nos. 1446–1450). It is apparent that they too succumbed to the testicular disease.

In Table I we present summarized post mortem findings on the animals which we have described above. When the animals were killed the right testis with its epididymis was dropped immediately into a fixing solution as a preparation for sectioning, the capsule of the testis being incised to hasten fixation. We used Bouin's fluid mainly, although we also used a formalin solution in some of the first work. The left testis was removed as soon as possible and the epididymis snipped across with sharp scissors and the cut surface smeared on a drop of Ringer's solution on a glass slide. This was examined immediately under the microscope. In turn, the left testis was trimmed clear from the epididymis and fatty tissue and weighed. In practically all cases the weights were much below normal. If one takes, for example, the first four animals of Table I it will be seen that the average weight of the trimmed left testis was approximately 0.8 gm. Donaldson (1) gives 2.694 gm. as a normal figure for the two testes of male rats of around 300 gm. in weight. This is a weight of 1.34 gm. each. The weights as recorded by us do not, however, give a

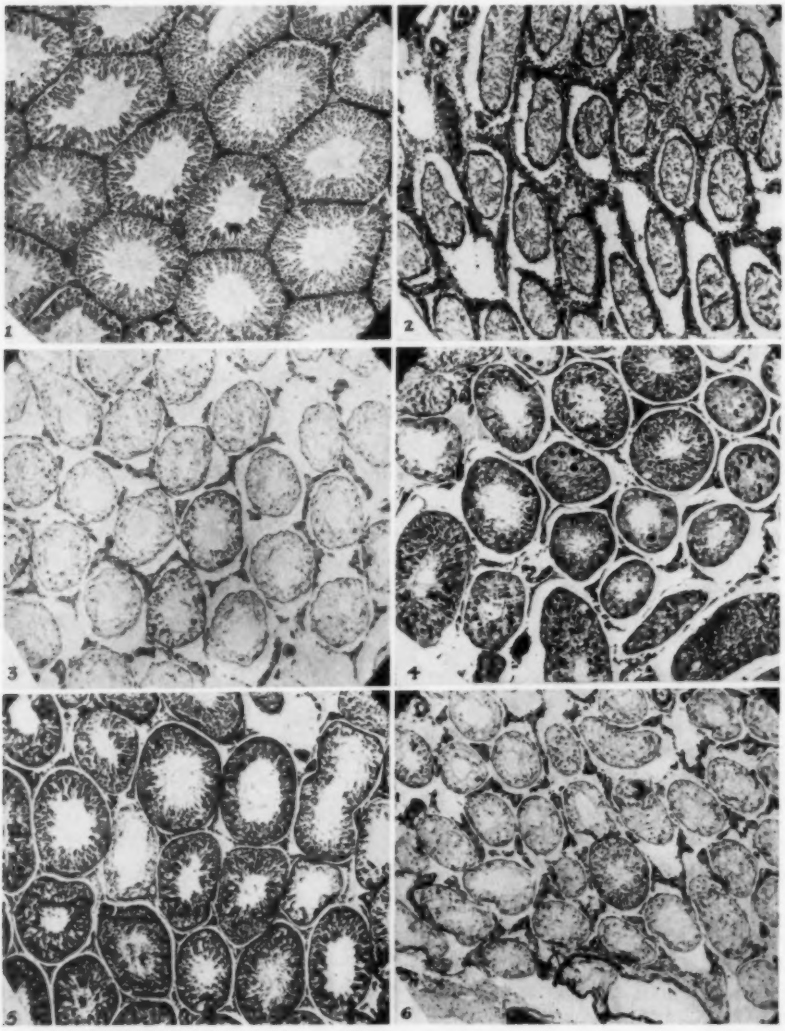

FIG. 1. Section from the normal testis of a male (M64) 43 weeks old. He was fed a good stock ration throughout. Acknowledgement is made to Dr. A. R. Lamb who supplied this section of a normal testis.

FIG. 2. Section from the right testis of No. 1263 (See Table I). Reduction in the size of the tubules and absence of germinal epithelium may be noted. Some of the shrinkage is probably due to the method of fixation.

[Legend continued on opposite page.]

complete idea as to the actual reduction in amount of tissue because of the very marked edema present. In some cases it was impossible to weigh the testis because in trimming off the epididymis the capsule of the testis was accidentally snipped and fluid oozed out leaving the testis almost flat. This edema may be noted in some of the figures presented.

With regard to the histological findings, our observations are not of sufficient variety to state what are the steps in the process of degeneration.* In most of the testes examined microscopically the end result is all that was apparent. This state is characterized by the complete disappearance of the germinal epithelium leaving only a loose syncytium of the sustentacular cells (Sertoli cells). The tubules are greatly reduced in size and are often flattened and misshapen. In very advanced cases the number of tubules is also much reduced and edema, in some cases very pronounced, is always present (See Figs. 1—6).

In a few of the testes of the first generation animals examined, the degeneration was not quite complete and in some of the tubules multinucleated giant cells were seen. In the excellent description of the testicular degeneration due to low vitamin E published by Mason (2) a similar "giant cell" stage has been described. The final disappearance of the germinal epithelium leaving only Sertoli cells is also a characteristic of low E male sterility. However, Mason did not apparently encounter the very marked reduction in the size of the tubules and the pronounced edema observed by us.

Discussion

The testicular degeneration which we have described above we believe to be unique in that it apparently is not caused by low vitamin E. In support of this view we would draw attention to our work presented in the immediately preceding paper showing that female rats on identical diets have

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Fig. 3. Section from the right testis of No. 1325 (Table I). Two tubules still possess some vestige of germinal epithelium.

Fig. 4. Section from the right testis of No. 1426 (See Table I). So-called "giant" cells may be seen in many of the tubules.

Fig. 5. Section from the right testis of No. 1427 (See Table I). Two tubules possessing practically no seminiferous epithelium can be seen. The other tubules are apparently normal.

Fig. 6. Section from the right testis of No. 1449 (See Table I). One tubule still retains its germinal epithelium while the others are degenerate.

All of the above photomicrographs were taken at a magnification of about 90 diameters. The original photos were reduced about one-half for reproduction.

Grateful acknowledgment is made to Dr. T. H. Bast of the Department of Anatomy who made the photomicrographs.

produced living young. Many of these females were litter-mate sisters of the males described above who produced litters long after the males were completely sterile. Poor reproduction was actually observed in these females, but, as we have shown, the reproductive mechanism failed in a manner entirely different from that occasioned by low E. If the testicular degeneration is due to low vitamin E, then there must exist an enormous difference in susceptibility between the two sexes, a difference which has not so far been noted by other workers.

Another point of interest lies in the fact that the male sterility occurring on the milk, iron, and copper diet is apparently intensified by the presence of the ferric chloride which we used as a supplement. In earlier work we studied the reproduction of males and females confined to a whole milk diet supplemented only with copper. We encountered no evidence of male sterility on these diets until we discontinued the animals. Because we were then aware of the sterility occurring in males on the iron and copper supplemented diet, we examined the testes of several of the former males. In one male kept for 46 weeks on the milk supplemented with copper, histological section of the testis showed almost complete degeneration. The brother of this animal, caged with him for the same length of time on the same diet, showed a practically normal testis. In other males kept for from 34 to 40 weeks on a copper supplemented milk diet the epididymal contents showed normal numbers and excellent motility of sperm. In two males on a similar diet after 51 weeks the testes were small and the smear from the cut surface of the epididymis of one showed only a few non-motile sperm. The smear from the other rat's epididymis showed a less than normal number of sperm, but there was excellent motility.

These observations indicate that indeed the testis may eventually be affected on the copper supplemented milk diets but not to the degree that occurs when iron as ferric chloride is further added to this diet. In the observations recorded in this paper the sterility was complete generally between 30 and 40 weeks on the latter diet. What the nature of the change caused by the iron is we cannot yet decide. We have already reported (3) a marked effect of adding ferric chloride to dry rations.

With regard to the record of No. 1426, it may be that the explanation lies in a variation in resistance of different animals. That his brothers did show marked testicular degeneration and that within his own testis some degenerate tubules were seen, lends support to the belief that he may have been affected earlier and then recovered. The possibility of recovery from such an advanced sterility, if caused by lack of vitamin E, would be small

according to Evans and Burr (4, p. 39). The iron dosage received by this group (0.5 mg. Fe per animal daily) was undoubtedly higher in the early weeks than that received by others fed 1.0 mg. Fe per 100 cc. of milk, since young rats do not usually consume 50 cc. of milk daily. Later, however, his iron intake was less than that of animals in other groups because as the rats near maturity as much as 100 cc. of milk may be consumed. It must also be mentioned that just prior to the time No. 1427 was discontinued, our milk supply was changed. Whereas it had been produced by cows receiving wheat straw as their only roughage, it was now supplied by cows receiving alfalfa hay and silage as part of their ration. We do not know whether or not this had a modifying effect on the course of the sterility, because of insufficient data.

As to the possibility in general of recovery from this sterility by means of a change in the diet, we cannot say anything definite. This, together with many other details of the problem, remains to be worked out.

Summary

1. Total sterility in male rats has been observed on a diet of cow's whole milk supplemented with small amounts of iron and copper salts. This sterility is characterized by the complete disappearance of the germinal epithelium, great loss in amount of testicular tissue, and pronounced edema.

2. This sterility is not due apparently to lack of vitamin E.

3. The effect on the testis is greatly intensified by some action of the ferric chloride. What this action is, is unknown.

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VITAMIN E IN IRON TREATED DRY RATIONS*

By

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IN 1928 we reported (1) the occurrence of sterility in female rats on mixed diets that had been treated with an ether solution of ferric chloride. The sterility was characterized by resorption of fetuses and was cured by adding daily doses of wheat germ oil to the diet of the affected females. We interpreted our results, therefore, as showing that the iron treatment had destroyed in some way the vitamin E *potency* of the diet. In this paper we wish to report further work which we undertook in an attempt to elucidate the manner in which the iron brings about this effect.

In addition to studying female performance, we have studied the effect of such a diet on the testes of males. The results secured from these experiments will also be reported below.

For a complete description of the ration which we used we would refer the reader to our earlier publication (1). It consisted of a mixture of 2/3 of a dry stock ration in use in this laboratory and 1/3 whole milk powder.[1] This mixture of stock ration and whole milk powder will be referred to throughout this paper simply as stock ration.

The iron treated ration was prepared by taking 99 per cent of stock ration and adding to it an ether solution of 1 per cent of ferric chloride. Sufficient ether was added to cover the ration in a flat dish. This was then set before a fan at room temperature and the mixture stirred at intervals until dry. Fresh batches of ration were made up every week or oftener.

MALE STERILITY ON THE IRON TREATED RATION

The work of Evans and Burr (2), Mattill and associates (3), and Mason (4) has shown that male rats become sterile when grown and maintained on diets lacking in vitamin E. The sterility is occasioned by a disturbance of

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[1] Klim, Merrell-Soule Company, Syracuse, New York.

normal spermatogenesis, such that at first non-viable sperm are produced and that later pronounced degeneration of the seminiferous epithelium takes place.

To determine if the iron treated ration would bring about sterility in males, as it had done in females, we took young male rats at weaning and fed them this diet. One group of seven received the iron treated ration alone; a second group of seven received the iron treated ration and in addition 200 mgs. wheat germ oil each daily (6 times per week). The first group was kept in one large cage provided with a screen bottom while the latter group was caged individually in screen-bottomed cages so that the wheat germ oil, which was fed in a separate dish, could be accurately measured to each.

The animals of both groups grew very well. Fifteen weeks after being started, the males of the first group averaged over 325 gm. while the males of the second averaged over 350 gm. Part of this difference was caused by an individual in the former which had an apparent infection of the respiratory tract and which weighed 262 gm. at the end of the 15 weeks. This individual also suffered from an everted penis (priapism?) which showed evident inflammation and scarring and hence he was discarded. His left testis weighed (trimmed) 0.33 gm.

After about 20 weeks on these diets the males were all placed in individual cages so that test matings with young normal females could be made. The females were of proven fertility and were placed with the males in their cages when in the early stages of estrus.

The mating performance of all the 13 males was very poor. A total of 44 opportunities to mate was given the six males remaining on the iron treated ration and only 9 positive matings were noted. The 7 males on the iron treated ration plus wheat germ oil had 54 opportunities to mate and 14 positive matings were recorded. One male of the first group and two of the second group never copulated, even though as many as 10 and 12 opportunities were afforded each of them. We were very much surprised at these results because it has been emphasized by Evans and Burr (2) that sex interest is maintained in males for very long periods even long after the fertilizing power has been lost.

From the nine positive matings by the males of the first group, no pregnancies resulted. From the 14 positive matings by males of the second group only 3 pregnancies resulted. This again was surprising since we had expected that, even though sterility would supervene in the males of the first group, those of the second group would be protected by the wheat

germ oil. An explanation doubtlessly lies in the fact that the oil which we used was one of low potency even though the dosage was relatively high.

Following these test matings all of the animals, except three in each group, were discontinued after approximately 26 weeks on the diet. The right testis from each animal was saved for histological study.

The three males from each group that were retained were placed in cages along with normal females and all were fed the stock ration. Here a true difference showed up between the two groups of males. From the females, caged with those that had received wheat germ oil, litters were secured at frequent intervals while no pregnancies occurred in the females caged with the males which had received only the iron treated ration. Later the males were exchanged between the two groups of females and again litters were born in the group which contained the males fed wheat germ oil while the other females produced no young.

These six males were later discarded and the right testes saved for histological study.

Sections from the testes of all the animals that had received only the iron treated ration showed complete or almost complete absence of germinal epithelium. Even in those three that had been maintained later for many weeks on the untreated stock ration, no histological evidence of recovery was noted. Marked edema was also noted in these testes. Six of the seven testes from the group which received wheat germ oil were sectioned and examined and all displayed an apparently normal structure.

It was apparent from these results that the iron treated diet was active in bringing about sterility in males. Those receiving this ration only were shown to be sterile after 20 weeks and those discontinued after 26 weeks were found to have lost practically all of the germinal epithelium from their testes. Even in those three that had been changed to the stock ration no evidence of testicular recovery was noted. It was, in fact, noted that their testes were flabby and edematous when they were discontinued. The trimmed left testis from one weighed only 0.73 gm.; that from the second weighed 0.90 gm.; while the testis from the third weighed 0.76 gm. Damage had been wrought beyond repair.

In contrast the testes from the group receiving wheat germ oil were histologically normal. The explanation of the poor fertility in this group during the test matings is probably the fact that the dosage of wheat germ oil was conferring only minimum protection. Evans and Burr (2, p. 39) have stated that there is a period of early infertility in males on low vitamin E in which the power to fertilize eggs is lost but that no structural

change may be noted in the testis. It is probable, therefore, that such a stage was reached in the males of this group when the test matings were first made. Those discontinued showed no histological evidence of degeneration while those changed to the stock diet completely recovered.

We cannot explain the lowered sex interest and activity exhibited by these two groups of males.

Further Experiments on Iron-Treated Diets

In our earlier work on female sterility brought about by iron treated rations, we observed a total absence of initial fertility. This point was of much interest in view of the fact that initial fertility, due to the reserves of vitamin E with which the animal is born, has been observed by Evans and his coworkers on nearly all rations studied by them. As a means of testing the iron treated ration further, and also to prepare sterile females for further experiment, we set about to raise many young females on this diet.

During the course of a month 94 females were taken at weaning and placed on the iron treated ration. Groups, containing for the most part 7 individuals, were kept in large cages fitted with screen bottoms. After 12 or 14 weeks, when the animals were well grown, stock males were placed in their cages so that mating might take place. At the same time vaginal smears were made daily on the females of seven of these groups so that accurate information could be obtained on their reproductive performance. Animals in which positive mating and implantation sign were noted, were weighed daily so that all criteria for the establishment of resorption were observed. Our experience was a repetition of what we observed earlier, *viz.*, that sterility was present in all females even in the first gestation. No young were born to any of these females, all the fetuses being resorbed. Those groups on which no vaginal smears were made were observed frequently to determine if any young were born, but none was found.

These observations, and many others which we made subsequently, permit us to state that females raised on the iron treated ration, prepared as we have described above, are invariably sterile by the time they reach maturity.

That the iron concentration of the ration was not of itself responsible for the sterile condition in the female rats, was indicated in our first work. There it was shown, and we have substantiated it in later work, that sterile females continued on the same diet but given in addition wheat germ oil (fed separately) produced young in the next succeeding gestation. It was

apparent that the effect was due to a change which the iron brought about in some constituent of the diet—the most likely of which was the fat. To determine if the iron would bring about the same result in the ration when added as a watery solution, and particularly where no drying was permitted, we performed the following experiment.

Sterile females from the groups discussed above were mated again and on the day of positive mating were segregated and fed the stock ration treated with ferric chloride dissolved in water. The same iron concentration was used as before but sufficient water was added so that the mixture after stirring was quite fluid in the feed cup. A fresh preparation was fed each morning, any food which remained from the day before being discarded. This method was followed throughout the period of gestation with the results indicated in Table I. There it may be noted that the females in which pregnancy was established bore living young and that the sterility

TABLE I

PERFORMANCE OF STERILE FEMALES ON STOCK RATION TREATED WITH AQUEOUS SOLUTION OF FERRIC CHLORIDE

Animal number	Diet	Date of mating	Placental sign	Young born
996	Stock ration treated with water solution of ferric chloride. Fed fluid daily. No drying.	1/20		Ovulates, 11 day cycle
1058		1/20	2/2	9 young
1102		1/22		Ovulates, 11 day cycle
1057		1/24		Ovulates, 12 day cycle
1090		2/5	2/18	7 young
1092		2/7	2/20	5 young (3 dead)
1080		2/13	2/26	6 young

was overcome. It was apparent, therefore, that drying with the accompanying aeration was in all probability a necessary factor in the destruction or inactivation of the vitamin E.

Other experiments performed later throw further light on this phase of the problem. As a means of determining if the iron treated ration could be prepared in a less expensive way by using less ether or else by using water as a solvent, we raised three groups of females from weaning to maturity on the following modifications of the ration. One group received the ration prepared by adding all the iron to 1/10 of the ration by means of ether solution. After this was dried before a fan it was intimately mixed with the untreated nine-tenths. The second group was fed a ration prepared

exactly as above except that the iron was added to the one-tenth of the ration by means of water solution. The third group received a ration prepared by adding the iron in water solution to the whole amount of the ration. The two rations which received the iron in water solution were finely ground after the drying process was complete. All three rations had the same iron concentration as the ration described above, *viz.*, 1 per cent of ferric chloride, and all were made up every week or oftener.

The three groups, each consisting of seven females, were mated after about 10 weeks on these diets. Vaginal smears were made daily on all the females.

Young were delivered by a majority of these females at the end of the first gestation, only a very few showing resorption. Second gestation performance, likewise, indicated a high degree of fertility although more resorptions were encountered than in the first gestation.

These results suggested that the sterility-producing effect of the original iron treated ration was evidently brought about by intimate contact of the iron and the ether-soluble material in the ration. This again directed our attention to the fats.

In addition to its effect on the vitamin E of a mixed ration, we have also found that iron will inactivate more concentrated sources of vitamin E. For example, we have treated wheat germ with an ether solution of ferric chloride and noted a very marked destruction or inactivation of the vitamin. Whereas the untreated wheat germ in daily doses of 250 mg. was found to bring about fertility in sterile females, raised and maintained on the iron treated ration, amounts four times as great (1.0 gm. daily) of the treated wheat germ did not prevent resorption of fetuses. (See Table II.)

Samples of wheat germ oil were also rendered partially impotent by long-continued aeration at 100° C. in the presence of iron. Fifty cc. of wheat germ oil were mixed with 100 cc. of ether containing 1 gm. of ferric chloride in solution. This mixture was placed in an oven at approximately 100° C. and heated air was bubbled through it for 10 hours. The ether soon evaporated leaving the iron distributed throughout the oil. Such treatment brought about very pronounced discoloration and the formation of some tarry residue which settled out. The aerated oil was poured through filter paper before being used. When fed at a level of 500 mg. daily during a gestation to sterile females which had been raised and kept on the iron treated ration, it did not prevent two of three females which became pregnant from resorbing again. (Table II.) The fact that one of the females produced young indicated that destruction or inactivation was not com-

plete. It may be noted that Evans and Burr (5) reported no deterioration in wheat germ oil aerated at 100° C. for 10 hours. The very marked inactivation in the wheat germ suggests that the increase in surface aids the reaction brought about by the iron.

TABLE II

THE EFFECT OF IRON TREATMENT ON WHEAT GERM AND WHEAT GERM OIL

Female number	Dietary change	Date of mating	Placental sign	Young born	Remarks
1097	250 mgs. wheat germ daily during gestation, fed separately.	1/15	1/29	8	5 dead.
1099		1/18	2/1	8	
1055		1/20	2/2	3	1 dead. Female died in parturition. 5 more young found in utero. Mature but dead.
1101		1/22			Ovulates, 11 day cycle.
1049		2/11	2/24	6	
1095		2/20	3/5	7	
1093	1 gm. iron treated wheat germ daily during gestation, fed separately.	2/28	3/13	0	Resorption.
1006		3/1			Ovulates, 12 day cycle.
1110		3/2	3/15	0	Resorption
1116		3/5	3/17	0	Resorption
1113		3/11	3/24	0	Resorption
1105		3/12	3/25	0	Resorption
1098	500 mg. wheat germ oil daily during gestation, fed separately.	2/5	2/17	10	
1075		2/7	2/20	5	
1076		2/8	2/21	?	Female dropped 41 gm. in wt. Small amt. blood noted around vulva. Young consumed?
1107		2/11	2/25	2	
1005	500 mg. iron treated wheat germ oil daily during gestation, fed separately.	2/21			Ovulates, 12 day cycle.
1111		2/21	3/6	6	
1096		2/22	3/8	0	Resorption
1084		2/23	3/6	0	Resorption
1106		3/7			Ovulates, 12 day cycle.

IS THE STERILITY PRODUCED BY THE IRON-TREATED RATION DUE TO THE PRESENCE OF AN "ANTIVITAMIN"?

In 1927 Evans and Burr (6) noted that certain fats and fatty acids (lard, hydrogenated vegetable fat,[3] oleic acid) when mixed with small doses of

[3] Crisco.

wheat germ rendered it impotent in the cure of sterility. They postulated the presence of antivitamin substances as an explanation (5). Their work, in conjunction with the experiments which we have described above, suggested to us the possibility that the sterility produced by the iron-treated ration might be due not so much to an actual destruction of vitamin E as to the production of some substance (or substances) which prevents vitamin E from acting. If such a substance was produced in the ration we thought that it might be possible to extract it. If it was extracted, the residue remaining might be able to support fertility. To test this point we performed the following experiments.

Two hundred fifty gram portions of the iron treated ration were extracted with ether at room temperature. The extraction was carried out by pouring several portions of ether over the ration in a medium-sized percolation funnel, the bottom of which was plugged with a wad of cotton. The dried residue, remaining after the extraction, was fed to sterile females beginning with the day of positive mating of the second gestation. This residue was fed *ad libitum* throughout the gestation to five females with the results indicated in Table III. Three of the females resorbed while two produced living young. These results, although not conclusive, suggested that, at least, the ration had been improved by the extraction and that the

TABLE III

THE EXTRACTION OF THE ANTIVITAMIN FROM THE IRON TREATED RATION BY MEANS OF ETHER

Female number	Diet	Date of mating	Placental sign	Young born	Remarks
1065	Females fed the ether	2/25	3/7	0	Resorption
1115	extracted residue from	2/25	3/10	0	Resorption
1003	iron treated ration *ad*	2/27	3/12	4	
1089	*libitum*.	2/27	3/12	9	
1103		3/6	3/18	0	Resorption
1122	230 gm. of stock ration	4/10	4/23	7	
1121	treated with iron-free	4/11			Ovulates, 12 day cycle.
1117	ether extract from 250	4/15	4/28	0	Resorption
1118	gm. of iron treated ra-	4/23	5/6	0	Resorption
1124	tion.	5/20	6/2	0	Resorption
1123	Iron treated ration, 250	4/10			Ovulates, 13 day cycle.
1126	mg. daily wheat germ	4/12			Ovulates, 13 day cycle.
1125	treated with iron-free	4/16	4/29	0	Resorption
1120	ether extract from 15	4/25	5/9	0	Resorption
1119	gm. of iron treated ra-	4/27	5/10	0	Resorption
	tion.				

ether extract might contain some of the antivitamin. We decided to test this by seeing if it would induce sterility when added to rations known to be amply supplied with vitamin E.

The reddish brown ether extract was found to contain large quantities of iron. This we decided must be removed so that no further iron effect could take place when it was added to other rations. The extract was, therefore, washed with several portions of 10 per cent HCl, since it was found that washing with water would not remove the iron. The acid washing was followed by two washings with water. The extract so treated was found to be practically iron free.

Sterile females raised and held on the iron treated ration were mated again and on the day of positive mating were segregated and fed the untreated stock ration to which had been added this iron free ether extract of the iron treated ration. Because the extract from 250 gm. of iron treated ration carried approximately 20 gm. of fat, we added this to 230 gm. of the stock ration. This was dried and stirred before a fan at room temperature and fed to the sterile females throughout the second gestation. The results, summarized in Table III, show that three out of four females that became pregnant resorbed again, indicating that the ether extract did carry some fertility-preventing substance.

In a further experiment bearing on the same point, other sterile females were fed during a second gestation a daily dose of wheat germ to which was added the washed extract from 15 gm. of iron treated ration. To 250 mg. of wheat germ in a small mortar was added the measured quantity of ether extract. This was placed before a fan until all the ether was dissipated. The resulting mixture of fats and wheat germ was offered daily to mated females receiving the iron treated ration. Some difficulty was encountered in getting the females to consume this addition but by removing the other food for a short time and in some few cases by mixing in a small amount of the dry ration, consumption was secured. The results, also presented in Table III, show that three females resorbed.

These findings indicated to us rather convincingly that one of the effects of the iron treatment on the stock ration was the production of an ether soluble substance which prevented the vitamin E of the stock ration or of the wheat germ from acting.

The Effects of the "Antivitamin" on the Vitamin E Reserves of the Animal

The results from the experiment described above, in which the dried residue from the ether extraction was fed to sterile females, were subject

to more than one interpretation. The fertility observed in two of the females might have been due to small amounts of vitamin E remaining in the extracted ration, or it might have been due to greater reserves in the animals' bodies, these reserves being unable to act in the presence of the antivitamin. To gain further knowledge of the effect of the antivitamin on the vitamin E stores of the body, we devised the following experiment.

A group of seven young females were placed at weaning on the stock ration to which had been added 5 per cent of wheat germ oil. They were allowed to remain on this high vitamin E diet for two weeks, so that their bodily stores might be increased. They were then fed the iron treated ration. After 8 weeks on the latter ration, a vigorous male was placed in their cage and daily vaginal smears were started. In the course of about a month all of the females mated and completed a gestation. All showed typical resorptions. Following this they were changed to a basal ration[3] and allowed to continue their reproductive performance for several gestations. In the second gestation all the females, except one, resorbed again. One female bore a litter of 4 young, one of which was dead. In the third gestation five of the seven females produced young and living litters were born to some females even in the fourth gestation. In the fifth gestation all the females that became pregnant showed resorption. We present in Table IV these results.

In another group of seven females this same performance was observed. These females received the iron treated ration for a period of 9 weeks after weaning and were then changed to the basal ration. After about 4 weeks on this basal ration, a male was placed in their cage and daily vaginal smears were made on the females. They, too, were permitted to go through several gestations with no change in diet. Their records are shown in Table IV (Nos. 1305 to 1311). There it may be seen that three of them resorbed in the first gestation but that two of these three produced living young in the second gestation before going on to resorb in the third gestation performance.

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[3] The basal ration had the following composition:

Casein (alcohol extracted and heated)	18
Dextrinized Starch	69
Cod liver oil	2
Salts 40	4
Yeast	7

This diet contains no known source of vitamin E. We have also shown it to be unable to support fertility by rearing young female rats to maturity and having them resorb during either the first or second gestation.

TABLE IV

THE INEFFECTIVENESS OF HIGH STORAGE RESERVES OF VITAMIN E IN THE PRESENCE OF THE ANTIVITAMIN SUBSTANCE

Female number	Diet	First gestation	Dietary change	Second gestation	Third gestation	Fourth gestation	Fifth gestation
1267	Stock ration, 95%	4/16 resorption		5/27 resorption	6/26 resorption	8/29 resorption	
1268	W. G. O., 5%	4/14 resorption		5/19 4 young (1 dead)	6/22 9 young	7/21 4 young	9/12 resorption
1269	*Fed 2 weeks*	4/12 resorption	Basal ration	5/19 resorption	(Mated but did not become pregnant)		
1270	Iron-treated ration after 2 weeks	4/12 resorption		5/21 resorption	7/28 4 young (1 partly consumed)	9/12 resorption	10/26 resorption
1271		4/18 resorption		5/24 resorption	6/28 4 young (partially consumed)	7/27 5 young	8/23 resorption
1272		4/24 resorption		5/30 resorption	7/1 8 young	8/5 resorption	9/6 resorption
1273		4/11 resorption		6/2 resorption	6/29 11 young (1 dead)	7/25 6 young	
1305	Iron-treated ration fed 9 weeks	6/18 9 young		7/19 12 young	8/17 resorption		
1306		6/6 resorption		7/29 8 young	10/14 resorption		
1307	Basal Ration after 9 weeks	6/17 9 young (all dead) female died					
1308		6/12 resorption		7/22 5 young	8/18 resorption		
1309		6/30 9 young		7/28 4 young	8/25 resorption		
1310		7/6 8 young (1 dead)		8/2 resorption	9/7 resorption		
1311		6/25 resorption		7/23 resorption	8/20 resorption		

These results, we considered, were of extreme significance. They clearly indicated that the antivitamin of the iron treated ration effectively inhibited the action of the vitamin E of the body. That these bodily reserves were high, especially in the first group, was shown by the later fertility, even after subsisting for some time on a basal diet known to be low in vitamin E. The results with the second group were entirely similar, except that their reserves were not so high, since they did not have the high vitamin E diet at the beginning. Whether or not the iron treatment *destroyed* the vitamin E of the ration, it apparently did not destroy the vitamin E reserves of the animal.

These results also indicated that the antivitamin was either stored in the body for some time or so affected the reproductive mechanism that it could not respond immediately after the removal of the antivitamin. This interpretation was supported by the fact that in the first of the above groups, nearly all of the females resorbed in the first succeeding gestation after being changed to the basal diet. Two of the females of the second group resorbed in the first gestation even when they had been on the basal diet for more than 4 weeks. As stated before, these two females subsequently produced living young.

In addition to the above considerations, it was apparent that resorption could not longer be considered proof of the depletion of vitamin E stores in the animal body.

Discussion

In this paper we have presented further data on the sterility-producing properties of a stock ration treated with an ether solution of ferric chloride. We have shown that males as well as females become sterile when confined for a sufficient length of time on such a ration and that such male sterility is absolute. Wheat germ oil conferred protection, at least to the extent of preventing any testicular degeneration, which was histologically demonstrable. The lack of fertility in the mating records of the group that received wheat germ oil we can explain only on the assumption that incipient sterility, such as Evans and Burr (2) describe in their stage A, resulted because of the rather impotent oil that we used. So far as our studies go, the male sterility which we observed was similar to that occurring on low E diets.

In further observations made on many females raised to maturity on this diet, we have found that sterility was always present and that no initial fertility was possible. This female sterility, we have been able to show, is due, in part at least, to the production of some substance (or substances)

that effectively prevents the action of vitamin E. For lack of a better name we have followed Evans and Burr's suggestion in calling it an "antivitamin." This material may be extracted from the ration by means of ether and can be used to counteract the vitamin E of other substances.

The question has arisen in our minds as to whether the iron brings about destruction of E in the ration as well as the production of the antivitamin. Thus, for example, does the iron treatment when applied to wheat germ destroy the E known to be present as well as build up the antivitamin? This question we are at the moment unable to answer.

The production of such a high concentration of the antivitamin in the iron treated ration makes one wonder if this substance is entirely absent from synthetic rations. Synthetic rations contain iron salts as part of their salt mixtures, and fats in some concentration are nearly always present. Our results with the iron treated rations, in which the iron was added to a part of the diet by means of ether or water solution, and in which the whole ration was treated with watery ferric chloride, suggest that to obtain the greatest production of the antivitamin, intimate contact between iron and lipoid material is necessary. It is probable that in basal rations as they are ordinarily mixed, such intimate contact does not take place, and that the antivitamin effect is of small moment. However, the possibility of such an effect taking place under conditions which we have described should not be overlooked.

It has been shown in the foregoing pages that the antivitamin does not apparently destroy the vitamin E of the body. It does, however, effectively prevent its action so that animals with large body stores of vitamin E show uniform sterility on the iron treated ration. It is of interest to note also that the antivitamin effect does not disappear immediately when animals are changed from the iron treated ration to a basal ration. Its effect has been noted for more than a month after the change in the diet. Whether this is due to a storage of the antivitamin in the tissues of the body or to some effect on the reproductive organs which prevents them from functioning normally, we cannot decide. It may be stated, however, that we have kept females on the iron treated diet for over a year and subsequently had them produce living young when changed to the untreated stock ration. It appears, therefore, that no permanent injury to the organs of reproduction is wrought by long continuance on the iron treated ration.

With regard to the effect of the iron treated ration on phases of the reproductive function, other than that involved in the death and resorption of fetuses, we cannot speak definitely. We have encountered poor ovula-

tory rhythm in many females raised on this diet. We have, however, also noted the same phenomenon on many females raised and maintained on basal rations. We have been unable to explain this on the basis of lack of vitamin B since both iron treated and basal rations contained what we considered to be ample amounts. Since Evans has emphasized the fact that in low E all other phases of the reproductive function are normal we are apparently denied the explanation that these disturbances are due to a lack of E in such a diet. Therefore, it may be that the presence of the antivitamin affects other parts of the female reproductive apparatus as well as its proved effect in interfering with the nutrition of the fetus.

Summary

Additional experiments carried out on an iron treated stock ration have shown that this ration also produces sterility in male rats reared on it.

In further work with female rats we have found that this ration induces 100 per cent sterility. There is no first litter fertility.

We have shown that the iron treatment brings about the formation of a substance which actively opposes vitamin E. This substance may be extracted from the iron treated ration by ether. It has been shown that even in the presence of high storage reserves of vitamin E, females are unable to utilize these reserves in the presence of the antivitamin. After being changed to a basal ration, these storage reserves may become effective after 4 to 6 weeks. It has been suggested that the antivitamin is stored for that time in the tissues or else brings about some change in the reproductive apparatus of the female that prevents it from nourishing implanted fetuses.

The significance of these findings is briefly discussed.

Addendum

Since the above manuscript was written, there have appeared several excellent papers by Mattill and associates in which they have carried forward their earlier work, (Jour. Amer. Med. Assoc. LXXXIX, 1505, 1927), on the phenomenon of auto-oxidation of fats and its relation to the destruction of Vitamins A and E. In a recent article (this Journal III, 421, 1931) they have definitely correlated the susceptibility to oxidation of several fats and oils and the reproductive behavior of rats fed diets containing these fats. Their work shows that oxidation in the fatty constituents of the diet destroys vitamin E and that this oxidation is quite extensive in certain common fats and oils. It follows, therefore, that the amount of vita-

min E in any diet is markedly influenced by the balance between anti- and pro-oxygenic substances. It is very probable that what we have termed anti-vitamin is pro-oxygenic in character. Regardless of what name is applied, it is apparent that it has a profound effect not only in the ration, but also in the body of the rat.

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CYSTEINE AND TAURINE AS SUBSTITUENTS FOR CYSTINE IN NUTRITION

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THE intermediary reactions of the amino acids in animal metabolism have been investigated largely by means of a few simple methods, apparently susceptible to straight-forward interpretation. Among these methods may be mentioned, attempts to detect intermediary metabolites in the urine after flooding the tissues with the amino acid in question by administering excessive doses *per os* or parenterally; investigation of the fate in the body, mainly by examination of the urine, of suspected intermediary metabolites; investigation of the fate of amino acids and their derivatives when perfused through surviving organs, generally the liver; *in vitro* treatment of amino acids by mild oxidizing and reducing agents, or by intracellular enzymes obtained from tissue extraction; biochemical study of certain peculiar metabolic abnormalities involving disordered amino acid metabolism. As Dakin (1) has pointed out, however, the obvious interpretation of the results of these methods may not be the true one in so far as the elucidation of normal metabolism is concerned. In particular, "the behavior of a substance when gradually produced, at low concentration and rapidly undergoing further change, may be very different from that of the same substance when rapidly injected in relatively high concentration into the tissues of an animal. Indeed, in the latter case it is always uncertain whether the substance ever really reaches the sphere of action in the cells normally concerned with its metabolism." The dangers of drawing close analogies between chemical reactions produced *in vitro* and those occurring in the body are obvious, while "it is always difficult to decide how closely reactions observed under pathological or abnormal conditions resemble those occurring in the normal organism."

The current facts and theories concerning amino acid metabolism are based largely upon the results of these methods of study, and the divergent theories coexisting in the current literature of this chapter in biochemistry may often be traced to divergent interpretations of the same set of data,or

to an emphasis upon one set of data to the entire exclusion of another set possessing an apparently contradictory significance.

The advent of a new method of attacking at least some of the same old problems is, therefore, a hopeful sign and offers some promise of being able to discriminate among the available data on amino acid metabolism between those that relate to normal metabolism and those that relate only to the particular set of experimental conditions under which they were obtained. By this new method, rations deficient in some one amino acid indispensable for growth are supplemented with possible or probable metabolic derivatives of the amino acid in which they are lacking. If the ration thus supplemented will support a more rapid growth than originally, or if it will support growth when originally it would not, it is evident that the amino acid derivative producing this increased efficiency is serving the unique functions of the amino acid itself, probably by conversion into it. Hence if, from other considerations, it is a probable normal metabolite of the amino acid to which it is chemically related, the probability of this being true is appreciably increased.

By this method, Cox and Rose (2) and Harrow and Sherwin (3) have shown that 4-imidazole lactic acid is apparently a complete substitute for histidine in metabolism. The latter investigators obtained evidence that 4-imidazole pyruvic acid could also substitute for histidine. It may be inferred from these results that histidine may undergo either oxidative or hydrolytic deamination. Similarly Jackson (4) and Berg, Rose, and Marvel (5) proved that tryptophane-deficient diets would promote growth when supplemented with indole-pyruvic acid.

Provided that the basal diet has been proven deficient in growth-promoting power, and that this deficiency has been related entirely to the amino acid in question, the significance of positive results obtained by this method is clear. However, the significance of negative results, in which supplements have failed to give evidence of improving the growth-promoting value of the basal diets, cannot be so definitely assessed. The supplement may depress the food intake of the experimental animals or be otherwise toxic to them, and yet be a normal metabolite and even under other conditions a successful substituent for the related amino acid. According to Dakin (1) it is "perfectly possible for substances of high toxicity to be constantly produced and rapidly transformed into other bodies, so that the relative concentration of the toxic substance is always low." Or the intake of food, both on the basal diet and on the supplemented diet, may be so low that a possible superiority of the supplemented diet cannot

be demonstrated except by an exact equalization of the intake of food of test and control animals, a refinement of the method that has not hitherto been attempted. The fact that Harrow and Sherwin obtained positive results with imidazole-pyruvic acid, which had yielded only negative results for Cox and Rose, and that Jackson and Berg, Rose, and Marvel obtained positive results with indole-pyruvic acid, which had yielded only negative results for Heft and Sherwin (6) constitutes objective evidence of the inconclusiveness of failures to obtain obvious responses to dietary supplements in this method of study.

Even if these possible interpretations of negative responses to dietary supplements in the method under consideration could be disposed of, the inability of the amino acid derivative to evolve a positive response in increased growth is still of uncertain significance, since it may be due, either to the fact that the derivative is not producible in metabolism from the amino acid to which it is chemically related, or that it is produced normally from the amino acid but by reactions which are all or in part irreversible, either under all conditions, or under the particular experimental conditions chosen, including the other chemical constituents with which the amino acid derivative is fed.

With respect to cystine, all attempts at substitution thus far recorded, except those involving dipeptides of cystine (7), have given negative results. Westerman and Rose (8) were unable to secure evidence of a successful substitution for cystine of dithiodiglycollic acid, β-dithiodipropionic acid, or α-dihydroxy-β-dithiodipropionic acid (9). The substitution of taurine for cystine in nutrition was apparently successful in experiments reported by M. L. Mitchell (10), but later attempts to confirm this work, reported by Beard (11), Lewis and Lewis (12), and Rose and Huddlestun (13) were unsuccessful. Lewis and Lewis obtained similar negative results with cysteinic acid. In another publication (14) Lewis and Lewis fed elemental sulfur to young rats receiving two types of cystine-deficient diets. In the words of the authors, "no evidence was obtained to suggest that sulfur can replace cystine or in any way alter the cystine requirements for growth in the young white rat." Although diglycyl-cystine and dialanyl-cystine are able, according to Lewis and Lewis (7), to promote growth when added to cystine-deficient diets, no indications were obtained that the dianhydride of dialanyl-cystine could successfully substitute for cystine.

Experimental evidence of the type discussed at the beginning of this article furnishes support for the belief that the first step in the catabolism of

cystine is its reduction to cysteine, representing the conversion from a diamino-dicarboxylic acid to a mercapto derivative of alanine. Lewis and McGinty (15), Lewis, Updegraff, and McGinty (16), and Rose, Shiple, and Sherwin (17) have shown that certain conjugated derivatives of cystine are in part excreted in the urine as the corresponding derivatives of cysteine, while the latter investigators have also shown that a conjugated cysteine derivative, phenyluraminocysteine, when injected into rabbits, is in part excreted in the urine as the corresponding cystine derivative. This agrees with other evidence in indicating that cystine and cysteine, as well as some of their derivatives, are interconvertible in the body, the conversion reactions being reversible.

In order to find out whether feeding experiments would confirm injection experiments in indicating a conversion of cysteine to cystine in metabolism the former was used as a supplement to a cystine-deficient diet in paired-feeding experiments with growing rats. Taurine was also tested in the same way, because the information on the value of this compound as a substituent of cystine in nutrition is divided.

Plan of Experiment

In both tests, the basal ration to which the supplements were added contained, as its main source of protein, dried skim milk powder to provide approximately 8 per cent of protein. According to numerous tests in this laboratory, such a ration when properly supplemented in other respects than protein is capable of supporting a fairly rapid rate of growth, but its growth-promoting value will always be increased when it is supplemented with cystine (18). The basal ration contained 20.41 per cent of dried skim milk, 4 per cent of the Osborne and Mendel salt mixture, 10 per cent of clear butterfat, 10 per cent of sucrose, 4 per cent of a cellulose flour[1] to furnish roughage, 1 per cent each of common salt, dried yeast (Northwestern Yeast Co.) and cod liver oil, and 48.59 per cent of starch. In the cysteine ration, 0.41 per cent of cysteine hydrochloride replaced an equal amount of starch, and in the taurine ration 0.50 per cent of taurine replaced an equal amount of starch.

The cysteine hydrochloride and the taurine used were obtained from the Eastman Kodak Company. The former contained 8.93 per cent of nitrogen as compared with the theoretical content, 8.89 per cent. The taurine contained 11.01 per cent of nitrogen as compared with the theoretical content,

[1] Cellu Flour, a product put out by the Chicago Dietetic Supply House, containing 37.8 per cent of crude fiber.

11.20 per cent. The cysteine hydrochloride was readily and completely soluble in cold water and in a saturated solution of sodium acetate. In the latter solution a heavy precipitate of cystine was produced by the addition of a drop or two of a dilute solution of hydrogen peroxide. It is evident that the cysteine hydrochloride was not contaminated with any appreciable traces of cystine.

In each of the two tests 8 pairs of rats were used; the rats in each pair were of the same sex, generally of the same litter, and were approximately the same in initial weight. They were kept in individual, cylindrical wire cages with raised bottoms. In the cysteine test, one rat of each pair received the unsupplemented basal diet and the other rat the same diet with a small amount of cysteine included. The food intake of each pair of rats was kept the same, and was so regulated that one or the other rat was kept close to the limit of its appetite. The food given to each rat was weighed daily on a fine chemical balance, and the daily allowance was increased regularly until one rat of the pair refused some of its allowance. In this event the daily allowance for both rats in the pair was reduced until the rat responsible for the reduction cleaned out his food dish. The allowance was then increased. The refused food was always left in the food dish, except at the end of an experimental week, when it was removed, weighed, and thrown out. In order to assure equal intakes of food for each week of the experiment, so that the weekly gains of pair mates would be directly comparable, increases in food allowances were not made on the last day of each experimental week. The taurine test was conducted in a similar manner.

Initial and final weights were the averages of three consecutive daily weights. Intermediate weights were also taken at the end of each experimental week. At the end of each test the rats were etherized and the body length, from anus to tip of nose, was measured, as an index of growth.

Experimental Results

The cysteine experiment was continued for seven weeks with the results shown in Table I. In each of the 8 pairs of rats, the rat receiving cysteine made the greater total gain, and attained the greater body length. The average difference in gain between pair mates is 18.1 grams favoring the cysteine rat. The standard deviation of the eight differences in total gain is 6.52 grams, the average being 2.78 times the standard deviation. For eight paired differences, according to the probability table of "Student," the odds are less than 1 in 10000 that chance alone would produce an average

result as great or greater than 2.78 times the standard deviation of the sample. The evidence may therefore be considered conclusive that cysteine will correct a cystine-deficient diet in the nutrition of growth. This means that it is convertible into cystine in the anabolic processes of growth. It may be inferred that this conversion takes place within the body rather than in the intestinal tract, since within the latter reducing rather than oxidizing conditions obtain.

In Table I, a nutritive index has been calculated according to the suggestion of Cowgill and Drabkin (19), by dividing the cube root of the body weight (grams) by the body length (centimeters). The larger this index becomes, the better the nutritive condition of the animal, as measured, for example, by its degree of fatness. In this experiment, the control rat possessed the greater nutritive index in 5 pairs, the cysteine rat in one pair, while in one pair, the mates were equal in this respect.

TABLE II

A COMPARISON OF THE WEEKLY GAINS OF PAIR MATES IN THE CYSTEINE EXPERIMENT

Week	Pair 1	Pair 2	Pair 3	Pair 4	Pair 5	Pair 6	Pair 7	Pair 8	Totals per week +	−	±
1	+	±	+	±	−	+	+	−	4	2	2
2	−	+	+	−	−	+	−	+	4	4	0
3	±	+	+	+	+	+	+	+	7	0	1
4	+	+	+	+	−	+	+	+	7	1	0
5	−	−	+	+	+	+	+	−	5	3	0
6	+	+	+	+	+	+	+	+	8	0	0
7	+	+	+	+	+	+	+	+	8	0	0
				Totals per pair					Totals for experiment		
+	4	5	7	5	4	7	6	5	43		
−	2	1	0	1	3	0	1	2		10	
±	1	1	0	1	0	0	0	0			3

Note: A + sign indicates a greater weekly gain by the cysteine rat.
A − sign indicates a greater weekly gain by the control rat.
A ± sign indicates equal weekly gains by pair mates.

In Table II, a comparison of the weekly gains of the pair mates in this experiment is made. For each pair and each week a plus sign (+) indicates a greater gain for the cysteine rat, a minus sign (−) a greater gain by the control rat, and a plus-or-minus sign (±) an equal gain by pair mates. The superiority of the cysteine-containing ration was evident from the third week to the seventh. The break during the fifth week in the accumulation of evidence favoring the cysteine ration was due to the fact that at this

TABLE I

THE EFFECT OF A CYSTEINE SUPPLEMENT TO A CYSTINE-DEFICIENT DIET ON ITS GROWTH-PROMOTING VALUE

	Pair 1		Pair 2		Pair 3		Pair 4	
	Control	Cysteine	Control	Cysteine	Control	Cysteine	Control	Cysteine
Final weight, gms.	135	143	128	141	132	160	144	158
Initial weight, gms.	52	50	53	52	50	52	40	41
Total gain, gms.	83	93	75	89	82	108	104	117
Final body length, cms.	17.9	18.5	17.8	18.6	18.3	18.8	18.1	19.2
Nutritive index	.287	.283	.283	.280	.278	.289	.290	.282
Total food, gms.	371	369	405	406	455	456	415	415
	Pair 5		Pair 6		Pair 7		Pair 8	
	Control	Cysteine	Control	Cysteine	Control	Cysteine	Control	Cysteine
Final weight, gms.	139	159	150	180	122	137	126	140
Initial weight, gms.	43	43	40	40	43	40	34	34
Total gain, gms.	96	116	110	140	79	97	92	106
Final body length, cms.	18.2	19.1	19.0	19.6	18.0	18.7	18.3	19.2
Nutritive index	.285	.284	.280	.288	.276	.276	.274	.270
Total food, gms.	363	366	481	481	417	413	414	414

time the cysteine ration used, newly made up, was found to contain considerably less than 8 per cent of crude protein, apparently due to an error in weighing out the ingredients. A new ration was immediately made up. In the entire experiment, 43 of the weekly comparisons favored the cysteine rat, only 10 favored the control rat, and 3 favored neither. This evidence is also clear cut.

The taurine experiment is typical of a negative outcome of a paired-feeding test, as the results in Table III clearly show. In only 2 of the 8 pairs of rats was the total gain of the taurine rat greater than that of its control, while the reverse was true in 6 pairs. The average difference between the total gains of pair mates was only 3 grams, favoring the control rat. It is so evident from inspection of the data that this difference possesses no statistical significance, that no analysis of it will be attempted. In body length and nutritive index, also the pair mates are not to be distinguished.

TABLE IV

A COMPARISON OF THE WEEKLY GAINS OF PAIR MATES IN THE TAURINE EXPERIMENT

Week	Pair 1	Pair 2	Pair 3	Pair 4	Pair 5	Pair 6	Pair 7	Pair 8	Totals per week +	−	±
1	+	+	±	−	+	±	+	+	5	1	2
2	−	+	−	−	+	+	±	−	3	4	1
3	−	−	−	−	−	−	−	−	0	8	0
4	+	+	±	−	−	+	±	−	3	3	2
5	+	−	−	+	−	−	−	+	3	5	0
6	+	+	−	−	−	−	+	−	3	5	0
7	−	+	±	+	−	+	−	±	3	3	2
	Totals per pair								Totals for experiment		
+	4	5	0	2	2	3	2	2	20		
−	3	2	4	5	5	3	3	4		29	
±	0	0	3	0	0	1	2	1			7

Note: A + sign indicates a greater gain by the taurine rat.
A − sign indicates a greater gain by the control rat.
A ± sign indicates equal gains by pair mates.

The comparison of the weekly gains of pair mates, as given in Table IV, is also clearly indicative of a negative outcome of the experiment. Of 56 possible comparisons, 20 favored the taurine rat, 29 the control and 7 were inconclusive.

As indicating the relative extent to which test and control rations would have been consumed if no restrictions had been placed upon food consump-

TABLE III

THE EFFECT OF A TAURINE SUPPLEMENT TO A CYSTINE-DEFICIENT DIET ON ITS GROWTH-PROMOTING VALUE

	Pair 1		Pair 2		Pair 3		Pair 4	
	Control	Taurine	Control	Taurine	Control	Taurine	Control	Taurine
Final weight, gms.	126	131	139	142	136	129	123	120
Initial weight, gms.	54	56	60	60	59	60	50	52
Total gain, gms.	72	75	79	82	77	69	73	68
Final body length, cms.	18.4	19.0	19.0	18.5	18.5	19.0	18.3	18.0
Nutritive index	.272	.267	.273	.282	.278	.266	.272	.274
Total food, gms.	409	409	437	435	434	434	430	429
	Pair 5		Pair 6		Pair 7		Par 8	
	Control	Taurine	Control	Taurine	Control	Taurine	Control	Taurine
Final weight, gms.	114	105	126	122	118	111	148	141
Initial weight, gms.	51	46	57	56	54	52	58	56
Total gain, gms.	63	59	69	66	64	59	90	85
Final body length, cms.	18.2	17.7	18.4	18.8	17.9	17.7	19.0	18.8
Nutritive index	.266	.267	.272	.264	.274	.272	.278	.277
Total food, gms.	367	367	451	451	402	402	475	477

tion, the number of times that test and control rats left food residues, thus causing a restriction in the amount of food offered to the pair, is significant. In the cysteine experiment, the control rats refused food 53 times and the cysteine rats 33 times. In the taurine experiment, the control rats refused food 42 times to 59 times for the taurine rats. It appears from these figures that the cysteine-supplemented diet was consumed somewhat more readily than the control diet, but on statistical grounds it may be doubted whether this is anything more than a fortuitous outcome.[2] In the taurine experiment it is even more evident that the taurine supplement did not significantly affect the avidity with which the basal cystine-deficient diet was consumed.

Conclusions

Cysteine added to a cystine-deficient diet will improve its growth-promoting value in animal nutrition. It is therefore convertible into cystine in anabolism.

No evidence was obtained indicating that taurine possesses this property.

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[2] In 86 refusals, the ideal outcome, if chance alone determined it, would be 43 favoring either control or cysteine rats. The actual outcome deviated from the ideal by 10 in each direction. The standard deviation of chance events of this type is equal to $\sqrt{0.5\times0.5\times86}=4.6$. The deviation from the ideal outcome is thus only slightly more than twice the standard deviation and might therefore have been due entirely to fortuitous factors.

UTILIZATION BY NORMAL ADULT SUBJECTS OF THE CALCIUM AND PHOSPHORUS IN RAW MILK AND IN ICE CREAM*†

By

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INTRODUCTION

IT IS generally recognized that a freely chosen American diet may not furnish adequate amounts of calcium and phosphorus, although it may give ample protein and fuel value. Milk is a good source of both calcium and phosphorus. Some studies have involved a comparison of calcium and phosphorus assimilation from various forms of milk available for animal and human nutrition. In no experiment has ice cream supplied most of the calcium and phosphorus required, although ice cream which is manufactured from milk and its by-products is used to such an extent that it is thought of as a typical American food. Much attention has recently been given to the influence of ultra-violet light upon calcium and phosphorus metabolism. Experiments here described were planned to study the comparative values of ice cream and raw milk as sources of calcium and phosphorus, using an acid-forming diet, as the American dietary tends to be acid-forming on account of the wide use of cereal and meat products. The subjects were normal adults, half of them receiving ultra-violet irradiations during the experiment.

HISTORICAL

There has been much discussion in the literature concerning the availability of calcium and phosphorus from raw and heat-treated milks. This has been reviewed by Willard and Blunt (1), including the work of Washburn and Jones (2), Magee and Harvey (3), Daniels and Loughlin (4), and Daniels and Stearns (5). Kramer, Latzke and Shaw (6) also dealt with this problem. These investigations point to the superiority of raw, evaporated, condensed, and quickly boiled milks over pasteurized and dried milks.

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Investigations have been conducted to study the relationships between ultra-violet light and the metabolism of calcium and phosphorus. Bethke, Steenbock and Nelson (7) and Hart, Steenbock and Elvehjem (8) found that ultra-violet light influenced the storage of calcium and phosphorus and the equilibrium of these elements in the blood of growing and of mature animals. Very few experiments have been conducted with normal human subjects. Hart, Tourtellotte and Heyl (9) found that an adult male on a calcium-deficient, acid-producing diet showed no special tendency to retain calcium or phosphorus when irradiated daily for 20 days. On the other hand, Burton (10) found high calcium and phosphorus retention for boys who received some ultra-violet irradiation while on a diet high in these minerals.

Blunt and Cowan (11) have reviewed most thoroughly the literature dealing with ultra-violet light in nutrition as well as the effects of the acid-base balance of the diet upon mineral metabolism. Finding the work on this latter problem full of contradictions, they cited particularly the work of Shohl (12, 13) which indicated that when no sudden shifts in acid-base balance took place the neutral diet promoted best calcium and phosphorus utilization for rats and apparently for normal children.

Experimental

The subjects of this investigation were 10 normal college women, five graduate students, and five under-graduates. None of the subjects had been taking cod liver oil previous to the experiment, and no one of them had been treated with ultra-violet light. Ordinarily the subjects had been spending little time out of doors, and during the experimental period this was particularly true. Five of the subjects were irradiated daily for a period of five minutes at a distance of 30 inches from the arc. The source of ultra-violet light was a quartz mercury vapour uviarc treater lamp,[1] operated at 110 volts on alternating current. The amount of irradiation given was considered suitable for the purpose and quite safe for the subjects, none of whom reported burning although there were mild effects upon the skin.

The experimental period of 18 days, begun the middle of October, was divided into six three-day periods. Two of these periods did not involve the collection of urine and feces as they were used for the subjects to become adjusted to their diets. The periods were as follows:

[1] Type R. T. Sp. 100, manufactured by the Cooper-Hewitt Electrical Company.

Period I	Preliminary; raw milk	Period IV	Preliminary; ice cream
Period II	Raw milk	Period V	Ice cream
Period III	Raw milk	Period VI	Ice cream

The diet for this study was acid-forming which, according to the literature, might be expected to influence the assimilation of calcium and phosphorus. All subjects were provided with a uniform amount of protein, fat, calcium, phosphorus, and sodium chloride. The caloric value of the diet was varied for the different subjects by means of cornstarch and sugar, so that body weights remained practically constant throughout the experiment. The calcium and phosphorus contents of the diet were calculated to be close to the minimum requirements for equilibrium so that differences in utilization would be more apparent. As a comparison was to be made of the availability of the calcium from ice cream and from milk, these foods supplied a large percentage of the calcium in the diet. Foods were selected which could be purchased and kept in quantity, so that the basal diet for the periods might be kept as nearly constant as possible.

TABLE I

FOUNDATION FOOD INTAKE IN GRAMS PER PERIOD

Food	Milk periods	Ice cream periods
Milk, raw	780	—
Ice cream	—	675
Apple	330	330
Bread	470	470
Meat	240	240
Orange juice	150	150
Oleomargarine	252	252
Prunes	150	150
Rice	180	180
Sodium chloride	3	3
Daily calories	1979	1979
Daily protein	58.2	55.3

The foods selected for the fixed diet (Table I) were ground lean round of beef, white bread, polished white rice, raw apple, orange juice, prunes, and oleomargarine. The required amount of beef, purchased at the beginning of the experiment, was freed of all gristle and bone, ground, thoroughly mixed, and weighed into individual portions. These individual portions were wrapped in oiled paper and kept at a temperature below freezing so that they were perfectly preserved until used. Rice, prunes, apples, and oranges were purchased in quantities to meet the needs of the

entire experiment. The bread, made in a uniform way, was secured fresh for each period from a local bakery.

The variables of the diet were ice cream and raw milk, each supplying approximately the same amount of calcium to the diet. The milk, obtained daily from the college dairy, was the product of several farm herds. Dairy products from this same source were used in the manufacture of the ice cream made according to current commercial practice. A large mix was made from butter, sweetened condensed skimmed milk, plain skimmed milk, sugar and gelatine, and contained 12.37 per cent fat, 10.62 per cent milk solids not fat, 15.00 per cent sugar, 0.40 per cent gelatine and 38.39 per cent total solids. The mix was processed by pasteurizing at 145°F. for 30 minutes, homogenized at 2500 pounds pressure, cooled at once to 40°F. and aged for 48 hours at 40°F. prior to freezing.

The rice, stoned prunes, oleomargarine, white bread, and salt were weighed each time in amount to supply the period requirement. The orange juice, pared and cored apple, ice cream and milk were weighed daily. Each subject prepared her own meals in the unit kitchens of the home economics building. The milk was consumed at the noon meal and was not heated. The ice cream, on account of its bulk, had to be served at both the noon and evening meals. Only distilled water was used for drinking and cooking purposes.

Aliquot parts of the milk were preserved daily with formaldehyde for analysis. A sample was taken from the ice cream mix for analysis, as the supply was uniform throughout the experiment. The rice was ground in a ball mill and analyzed separately. The food composite was composed of one-third the weight of the individual food intake of orange juice, white bread, stoned prunes, ground beef, and apple for all the periods. The residue from the oleomargarine was found to be negligible and was not analyzed. Great care was taken to insure uniform sampling of food. The composite was dried to constant weight at 80°C. in an electric oven, pulverized, and stored in tightly covered containers.

Careful collections of all feces and urine were made for Periods II, III, V, and VI, carmine being used to mark the feces of the different periods. The urine was measured, specific gravity was taken, and samples were saved for analyses. The urine was preserved with toluene. The feces were dried at a low temperature to a constant weight, weighed, pulverized and saved for analyses.

Determinations of calcium in the food, feces, and urine were made by the McCrudden Method (14) from samples ashed in a muffle furnace. For

the phosphorus determinations the volumetric method given in the Official and Tentative Methods of Analysis by the Association of Official Agricultural Chemists (15) was used, magnesium nitrate being used in ashing.

The dietary calculations for calories and protein and the preliminary estimations of calcium and phosphorus contents of the diet were made from the tables of Rose (16). The food intake per period and the acid-base balance of this food as estimated from the tables of Sherman and Gettler (17) and Forbes (18) are indicated in Table I and Table II. The diet was acid-forming to the extent of 42 cc. of normal acid per period, without the prunes, for which no figure was available.

TABLE II

EXCESS OF ACID-FORMING OR BASE-FORMING ELEMENTS IN THE DIET PER PERIOD

Article of food	Grams of food	cc. of N acid or base per 100 grams		Total cc. acid	Total cc. base
		acid	base		
Apple	330		3.76		12.41
Bread	470	7.10		33.37	
Meat	240	13.91		33.38	
Raw milk	780		2.37		18.49
Orange juice	150		5.61		8.42
Oleomargarine	252				
Prunes	150				
Rice	180	8.10		14.58	
Ice cream	675				
Total				81.33	39.32

Note: The basicity of the ice cream was considered equivalent to that of the raw milk. Prunes also contribute to the acid-forming effect of the diet.

EXPERIMENTAL RESULTS

Table III gives the calcium and phosphorus content of the diet per period, as determined from analyses of all foods. The calcium and phosphorus contents of the raw milk and the ice cream and also the per cents of total calcium in the diet contributed by the latter two foods are given. The raw milk and ice cream furnished more than three-fourths of the calcium. The compositions of milk and ice cream are such that they necessarily furnished smaller proportions of the phosphorus, amounting to a little more than one-third of the total.

The figures for total intakes, output in feces and urine and the balances for calcium and phosphorus are given in Table IV. For comparison, balances were calculated for each period in grams per kilogram of body weight.

It is clear that the total intake of calcium was kept near the minimum required for maintenance in adults, as had been planned, so that differences might be evident.

For three of the irradiated and four of the non-irradiated subjects, the calcium balances for the ice cream periods were higher than for the fresh milk periods. Three subjects of each group showed better phosphorus balances for the ice cream periods.

Though well aware that averages from a small number of cases are not conclusive, it seemed of interest to compare the average calcium and phosphorus balances in grams per kilo per period, as follows:

Calcium			Phosphorus		
	Milk	Ice cream		Milk	Ice cream
Irradiated subjects.....	−0.004	−0.005	Irradiated subjects.....	+0.011	+0.012
Non-irradiated subjects...	−0.003	−0.001	Non-irradiated subjects.	+0.018	+0.018

Each figure is the average for the five subjects in that group. For the non-irradiated subjects, the calcium of ice cream gave the more favorable balance, while for irradiated subjects, the calcium of fresh milk seemed slightly better. The non-irradiated subjects showed calcium balances at least as favorable as those of the irradiated subjects. The non-irradiated subjects also showed somewhat better phosphorus balances than the irradiated subjects, but practically no differences appeared between the milk and ice cream periods.

It is evident that the calcium from ice cream, containing condensed sweetened skim milk, was at least as well utilized as the calcium of raw milk, when furnished at a low level of calcium intake to normal adults on a diet somewhat acid-forming. More advantageous calcium utilization was not found in the group receiving ultra-violet irradiations, under conditions as described. As the compositions of milk and ice cream are such that they necessarily furnished smaller proportions of the total phosphorus in the diet, the results for phosphorus metabolism are less convincing although apparently similar to those for calcium.

Summary

A metabolism study was made to compare the utilization by 10 normal adult subjects of the calcium and phosphorus in raw milk and ice cream. The diet was somewhat acid-forming as is common throughout this country. Five of the subjects received ultra-violet irradiations daily.

TABLE III
CALCIUM AND PHOSPHORUS IN THE DIET

	I		II		III		IV		V		VI	
	Ca	P	Ca	P	Ca	P	Ca	P	Ca	P	Ca	P
Grams for period	1.252	1.984	1.252	1.984	1.252	1.984	1.322	1.900	1.328	1.978	1.328	1.978
Grams from raw milk	0.984	0.792	0.984	0.792	0.984	0.792						
% from raw milk	78.59	39.92	78.59	39.92	78.59	39.92						
Grams from ice cream							1.060	0.766	1.060	0.766	1.060	0.766
% from ice cream							80.18	40.32	79.82	38.73	79.82	38.73

The raw milk contained 0.126 per cent calcium, and 0.099 per cent phosphorus.
The ice cream contained 0.157 per cent calcium, and 0.133 per cent phosphorus.

TABLE IV
CALCIUM AND PHOSPHORUS BALANCES IN GRAMS PER PERIOD (AVERAGES OF PERIODS USED)
SUBJECTS IRRADIATED

Subject, weight	Dairy product	Calcium				Phosphorus			
		Intake	Output	Balance	Balance per kg.	Intake	Output	Balance	Balance per kg.
L.D.	Milk	1.252	1.547	−0.295	−0.005	1.984	1.922	+0.062	+0.001
60.7 kilos	Ice Cream	1.328	1.550	−0.222	−0.004	1.978	1.747	+0.231	+0.004
A.G.	Milk	1.252	1.448	−0.196	−0.004	1.984	1.312	+0.672	+0.014
49.3 kilos	Ice Cream	1.328	1.414	−0.086	−0.002	1.978	1.235	+0.743	+0.015
I.G.	Milk	1.252	1.658	−0.406	−0.008	1.984	0.846	+1.138	+0.023
50.5 kilos	Ice Cream	1.328	1.628	−0.300	−0.006	1.978	1.048	+0.930	+0.018
M.P.	Milk	1.252	1.324	−0.072	−0.001	1.984	1.811	+0.173	+0.003
60.9 kilos	Ice Cream	1.328	1.644	−0.316	−0.005	1.978	1.450	+0.528	+0.009
L.R.	Milk	1.252	1.466	−0.214	−0.004	1.984	1.269	+0.715	+0.014
50.0 kilos	Ice Cream	1.328	1.783	−0.455	−0.009	1.978	1.368	+0.610	+0.012

TABLE IV (continued)
SUBJECTS NOT IRRADIATED

Subject, weight	Dairy product	Calcium				Phosphorus			
		Intake	Output	Balance	Balance per kg.	Intake	Output	Balance	Balance per kg.
F.D.	Milk	1.252	1.381	−0.129	−0.002	1.984	0.894	+1.090	+0.020
55.7 kilos	Ice Cream	1.328	1.154	+0.174	+0.003	1.978	1.129	+0.849	+0.015
E.H.	Milk	1.252	1.280	−0.028	−0.001	1.984	0.808	+1.176	+0.022
54.3 kilos	Ice Cream	1.328	1.677	−0.349	−0.006	1.978	0.963	+1.015	+0.019
N.K.	Milk	1.252	1.610	−0.358	−0.005	1.984	1.140	+0.844	+0.012
72.9 kilos	Ice Cream	1.328	1.542	−0.214	−0.003	1.978	0.906	+1.072	+0.015
N.S.	Milk	1.252	1.166	+0.086	+0.002	1.984	0.916	+1.068	+0.021
50.6 kilos	Ice Cream	1.328	1.193	+0.135	+0.003	1.978	0.884	+1.094	+0.022
R.W.	Milk	1.252	1.792	−0.540	−0.011	1.984	1.264	+0.720	+0.014
50.9 kilos	Ice Cream	1.328	1.503	−0.175	−0.003	1.978	0.990	+0.988	+0.019

No special advantages in the utilization of calcium and phosphorus seemed to be conferred upon those subjects who received ultra-violet irradiations, as described, throughout the 18 days of the experiment.

Four of the non-irradiated and three of the irradiated subjects showed more favorable calcium balances when ice cream was the chief source of calcium. Normal adult subjects on an acid-forming diet utilized the calcium from ice cream made with condensed milk at least as well as the calcium of raw milk. In general the phosphorus balances followed the trend of the calcium balance figures.

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VEGETABLES IN THE DIETS OF PRESCHOOL CHILDREN*

By

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VARIETY AND FREQUENCY OF VEGETABLES IN THE WEEKLY DIETARY

AMERICAN families eat more vegetables than they did ten years ago. The increased consumption is reflected in quantities shipped from the larger markets. For example, the consumption of three vegetables as indicated by shipments from eleven markets more than doubled in six years.[1]

		1920	1926
Cauliflower	Crates	2,343,000	5,550,000
Lettuce	Crates	7,928,000	17,236,000
Spinach	Tons	49,600	119,200

This change in the family diet together with the more marked attention of the past five years to the habits of the preschool child have affected his diet.

Before he cuts his teeth the child is given specially prepared vegetables. The demand for pulped and strained vegetables has been great enough to bring many commercial products on the market. At what age the young child may have any or all vegetables from the family table is still an unsettled question. That between three and five years he is getting a wide variety is made evident through surveys of typical groups. (See Table I)

The variety of vegetables and the frequency of serving them to preschool children are brought out in Table I which presents data gathered by keeping daily records of the food of 42 children for periods varying from one to four weeks as shown in Table II. As indicated in Table II, none of the summer and early fall months when fresh vegetables are most plentiful was included.

* This study was made possible through the co-operation of Professors Thomas Vance, Lulu Lancaster, and Lydia Swanson of the Department of Child Development.

[1] Figures from the U. S. Dept. Agr. Yearbook, 1926, p. 956.

TABLE I

AVERAGE NUMBER OF SERVINGS OF VEGETABLES PER CHILD PER WEEK

Group	I	II	III	IV	V	VI
Asparagus	.1	.0	.0	.2	.9	2.6
Beans, snap	1.0	1.8	2.0	1.7	1.9	.6
Beets	.3	.6	.3	.0	.6	.4
Cabbage	.6	1.3	.2	1.0	1.6	.7
Carrots	1.6	2.4	1.4	3.0	2.1	2.7
Cauliflower	.1	.2	.0	.0	.1	.5
Celery	.9	1.2	.6	1.0	1.2	.6
Corn	.2	.5	.0	.2	.3	.4
Lettuce	2.0	3.2	4.9	1.5	3.3	2.5
Onions	.0	.3	.1	.2	.4	.2
Parsnips	.2	.0	.0	.0	.1	.0
Peas	1.4	2.1	1.6	1.2	2.1	1.7
Spinach	.8	1.0	1.8	1.3	1.3	1.0
Squash	.1	.1	.0	.0	.0	.0
Sweet potatoes	.5	.4	.0	.0	.0	.0
Tomatoes	1.5	1.4	2.4	1.2	2.4	2.4
Turnips	.3	.0	.0	.5	.7	.0
Potatoes	6.1	6.9	7.4	6.7	—	—
Others and mixtures	1.5	.9	1.1	3.0	.9	.1
Total excluding potatoes	13.1	17.4	16.2	16.0	19.9	16.4

TABLE II

TIME OF SURVEYS AND CHILDREN INCLUDED

Group*	Season	Duration of survey wk.	Children		Age		
			Total no.	Girls no.	Average yr.-mo.	Min. yr.-mo.	Max. yr.-mo.
I	Nov.–Dec.	4	6	2	4– 5	3– 2	5–3
II	Nov.–Dec.	4	7	3	3– 8	2–11	4–8
III	Jan.–Feb.	1	13	7	4– 2	3– 3	5–0
IV	Jan.–Mar.	1	6	3	4– 1	3– 8	4–8
V	Apr.	2	5	3	4– 6	4– 0	5–0
VI	Apr.–May	2	5	2	3–11	3– 8	4–2

* The children of Groups I and VI did not attend a nursery school.

These children are receiving more than two servings of vegetables per day in addition to a serving of potatoes. Nine or more different vegetables are served per week to the average child without taking into account the

different ways of preparing them for the table. Lettuce, carrots, and tomatoes are most commonly used, with peas and snap beans next.

In 1911 Griffith (3) advised that carrots, parsnips, squash, stewed tomatoes and turnips be given with extreme caution to children of three to six years. He advised avoidance of cabbage, cauliflower, raw celery and raw tomatoes. Most of them are common foods in the child's diet now.

Nutritive Value of Vegetables in the Mixed Diet of Children

Because in many cases the servings of vegetables are very small, an individual dietary study of six nursery school children was carried out to determine what contribution vegetables make to the diet, especially in minerals.

The children chosen seemed representative of a large group though they may have fared better than the average child. They were in good physical condition as indicated in Table III. Their mental ratings by the nursery school psychologist were above the average for their ages. The parents were high school or college graduates, the fathers professional or business men and the mothers interested in feeding their children according to the best standards they knew.

Table III

Description of Subjects

	Sex	Age yr. mo.	Height in.	Weight lb.	Deviation from average* per cent
Teddy	Boy	3- 8	41.8	45.0	+18
Buster	Boy	3-10	39.0	34.0	−3
Reed	Boy	4- 5	43.6	44.8	+4
Bev. Ann	Girl	4- 5	41.4	42.0	+11
Persis	Girl	4- 6	41.5	38.2	0
Cornelia	Girl	4- 8	44.1	41.2	0

* Average weight as given by Woodbury.

Samples for analysis. All foods eaten by each child during a week were weighed and duplicate samples were collected. Vegetables, including potatoes, as free as possible from butter, cream or thickening, were kept separately. Milk samples were preserved with formaldehyde and kept in a refrigerator. All other foods were dried. Composite thoroughly mixed samples representative of a week's food for each child were secured for analysis. The water intakes for the week were measured.

Quantities of foods. Vegetables including potatoes, composed a significant portion of the diet. By weight as served they constituted one-seventh of the total mass of food, milk for drinking three-sevenths, and all other foods three-sevenths. The maximum quantity of vegetables as consumed by one boy was nearly three times the minimum eaten by the oldest girl. Their variety is shown in Table I, Group IV. The vegetables contained on the average 91 per cent water; other foods aside from milk contained 67 per cent water.

That the energy values of the diets of these children approximate the average for their age (5) is indicated in the last column of Table IV. The quantities of milk as shown in Column 3 appear small but these volumes do not include that incorporated in foods.

TABLE IV
AVERAGE FOOD CONSUMPTION PER CHILD PER DAY

	Vegetables as served	Servings of vegetables	Beverages		Total energy**
			Milk	Water	
	oz.	no.	cups*	cups*	Cal.
Teddy	6.8	2.4	1.7	0.6	1454
Buster	9.2	4.1	2.6	1.8	1577
Reed	8.2	3.8	3.2	1.0	1704
Beverly Ann	6.7	3.0	1.4	1.4	1429
Persis	5.1	2.4	1.8	1.9	1272
Cornelia	3.3	2.8	2.7	2.0	1235
Average	6.5	3.1	2.3	1.5	1445

* 237 cc.

** Calculated on basis that 145 gm. milk yield 100 Cal. (8) and one gram of a dried mixed diet yields 5 Cal. (2).

Methods of analysis. To determine the contribution of vegetables to the total minerals of the diet, all foods were analyzed as follows: for total ash by igniting samples in a muffle furnace at a temperature of about 400°C; for calcium by McCrudden's method (7), titrating the oxalate against standard permanganate; for phosphorus by Neumann's method (4), dissolving the phosphomolybdate in alkali and back titrating with standard acid; for iron by Kennedy's colorimetric method (6), extracting the sulphocyanate in isoamyl alcohol. Analytical data on water as reported by the State Experiment Station were used. For the iron content of milk, an average composition figure as reported by Sherman (8) was used.

Vegetables as a source of minerals. That the vegetables were comparatively high in minerals is shown by averaging the figures for the dried products for these six children (See Table V). The total solids of vegetables are higher in ash and in some individual minerals than is the dried product from the mixture of other solid foods in the child's diet. A liberal mineral intake may be secured by a liberal use of vegetables.

TABLE V

AVERAGE PERCENTAGE COMPOSITION OF DRIED FOODS

	Dried vegetables	Other foods, dried
Total ash	7.51	2.93
Calcium	0.21	0.16
Phosphorus	0.30	0.23
Iron	0.0073	0.0053

From the total food intake for the week and the percentage composition of the foods the average daily mineral intake of each child was calculated and is shown in Table VI. With the exception of the child who drank less than one and one-half cups of milk per day, the diets of these children were adequate in minerals according to commonly accepted standards.

TABLE VI

AVERAGE DAILY MINERAL INTAKE PER CHILD

	Total ash	Calcium	Phosphorus	Iron
	gr.	gr.	gr.	mg.
Teddy	9.50	0.98	1.00	13.3
Buster	10.58	1.20	1.26	14.3
Reed	11.83	1.47	1.48	13.8
Bev. Ann	7.74	0.85	0.86	13.1
Persis	8.82	0.91	0.98	16.9*
Cornelia	9.83	1.20	1.08	8.5
Average	9.72	1.10	1.13	13.3

* Liver eaten on 3 days of week.

The influence of milk on the total mineral intake is noticeable when the average percentages of the total food nutrients contributed by the vegetables in the diet are calculated and compared with those of milk (See Table VII). For calcium and phosphorus, the milk drunk is the principal

contributor and it furnished about four-tenths of the total minerals. The chief value of vegetables so far as these figures show lies in their comparatively high iron content. They furnished more than one-sixth of the total

TABLE VII
AVERAGE CONTRIBUTION OF FOODS TO THE MINERAL INTAKE

	Vegetables	Milk	Other foods
	per cent	per cent	per cent
Ash	2.3	42.1	54.7
Calcium	5.6	65.4	26.1
Phosphorus	8.3	52.2	39.5
Iron	17.3	9.8	72.4

iron intake. Just as the diet of the child receiving little milk was low in calcium and phosphorus, just so the diet of the child who ate only half as much vegetables as the average was far the lowest in iron. Hence, it is important that vegetables be used to a great extent in diets like those of the preschool child, which usually contain much milk.

Reasons for liberal use of vegetables. Besides contributing much iron and some other minerals, the vegetables furnished approximately 10 per cent of the total energy for these children and acted as a vehicle for butter and cream. The texture of the diet and its vitamin content, especially that of the antiscorbutic vitamin, are probably determined to a large extent by the vegetables.

But for another reason the vegetable content of the diet is important. As the child grows older, the quantity of milk in the diet remains stationary or even decreases while the consumption of other foods increases to meet the growing energy need. Among these foods the proportion of those rich in minerals and vitamins needs to be increased if the quantities necessary for growth and regulation of body processes are to be maintained. Perhaps nothing helps more in developing a liking for a particular food than does its early introduction into the diet. An appetite for a variety of vegetables may be developed during the preschool years, a transition period following infancy.

APPETITE OF PRESCHOOL CHILDREN FOR VEGETABLES

Much attention has been given during the past few years to the appetite of children because of many reports showing the prevalence of anorexia. Aldrich thinks that this lack of appetite is a late development for he says

(1) that the general practitioner of thirty years ago would be incredulous were he told that the biggest feeding problem today is to get the child to eat the foods he should. Physiological, hygienic or psychological factors may explain lack of appetite. Sometimes the influencing factor is dietary. We know little about the reasons for children reacting favorably to some foods and at the same time refusing to eat others equally desirable from the viewpoint of the adult. Several observers have said that vegetables are the least liked of all foods by children.

To get definite evidence of the attitude of young children toward vegetables, the nursery school at the noon hour presents the best opportunity because many children can be observed at the same time and under the same conditions.

Comparison of nursery school children and other preschool children. That the reactions of the nursery school children are representative of preschool children is supported by comparison of the ten children composing groups V and VI of our survey (p. 116.) They were observed at all their meals for two weeks; after the first meal the mothers recorded on mimeographed forms the responses of the children to vegetables as indicated by reactions to be designated later (p. 123). Comparison is made only between attitudes toward vegetables eaten at home. The nursery school children had a total of 145 servings of vegetables at home, the other group 164. In each group there was a definite liking for a vegetable manifested toward 77 per cent of the servings. The nursery school children liked 98 per cent of the raw vegetables served, the other group 85 per cent. The former group disliked a somewhat greater percentage of all the vegetables (10 versus 4 per cent) but there were included two children who refused to eat at home some vegetables they ate at school; one child announced that his mother's vegetables were black and those at school white. The order of eating vegetables among foods served was the same. The cooked products were not standardized and the judgments of mothers on the attitudes of their own children might be biased. However, this was no more likely to be true in one group than in the other. On the whole the two groups were very much alike in their responses.

For the main part of the appetite experiment, two workers observed six groups of nursery school children at their noon meal for periods five weeks long. The 55 children observed varied in age from 1 year 11 months to 4 years 10 months. Among these children it was an exceptional occurrence for a child not to appear to want to eat its lunch.

Of course conclusions reached after observing a small number of children

in a single community are tentative and can be regarded only as preliminary. Our results are recorded here in the hope that others may be interested in this aspect of nutrition. Though we may know just what foods should make up a child's diet, whether he eats certain ones in sufficient quantity appears to depend very much upon his attitude toward them.

Method of determining desire of average child. For the first two groups of 15 and 13 children, records were kept of all remarks concerning vegetables, of the order of eating vegetables relative to other foods served and also of the apparent desires of each child for the vegetables and for the meals as shown by his manner in eating them. To have a method of designating differences in desire for a food by various children and for various foods by a single child numbers were used as follows: 1 for great desire; 2 for a liking, 3 for indifference; 4 for a dislike; 5 for great repugnance. The same system was used to record the degree of apparent appetite of each child for his meal. It was thus possible to secure averages. An element of personal judgment enters in but two observers agreed well on any cases that were compared.

Two other groups of children were served, during their five-week period, twelve vegetables which were wholly new to them. So far as possible the methods of preparation of the vegetables were standardized. The service was regulated by giving very small portions and increasing them somewhat after the vegetable had been served twice. The position of the new vegetable on the plate was controlled by putting it at the child's left with the sandwich on the far side of the plate and the meat or egg near him. Records were kept of order of tasting and finishing the vegetable, of verbal remarks and of facial expressions that were inspired by vegetables. Remarks and facial expressions were classified into three types: interest or curiosity as "What is this?"; definite favor as "I like this" or "This is good"; definite displeasure as "This is nasty" or "I don't like this." No attempt was made to grade intensity of response by a child. Using the number of interest, favorable and unfavorable responses the vegetables were ranked and a total score for each secured; the procedure was repeated with facial expression indications.

Appetite as shown by manner of eating. The attitudes of the first group toward creamed turnips and toward a mixture of spinach and chard were indicated by an average figure 3 for each; they were eaten as a matter of course. Toward the other 11 common vegetables served a total of 22 ways there was noticeable desire. In general, the appetite of the second group for vegetables checked that of the first; they were not served creamed tur-

nips nor the spinach-chard mixture but their attitude towards creamed onions is shown by an average between 2.5 and 3.0, close to indifference. Except for cooked, strong-juiced ones, these 28 children liked common vegetables.

Appetite for vegetables as shown by remarks. While new vegetables were served 56 remarks of definite favor and 55 unfavorable remarks were overheard showing neither a great liking nor dislike for new vegetables. There were 228 verbal interest remarks made. Among these were 36 comparisons of the forms of the vegetables to familiar objects, as when broccoli flowerets were served, "My oak tree is all gone" or about French endive, "Is this celery?" Such remarks show interest among children in the appearance of their food. There were 10 interest remarks comparing the flavor of the vegetable to that of a familiar one. Among the vegetables fed both groups the final scores were not very different. Brussels sprouts and raw kohlrabi were liked best. No single property of a vegetable marked it for favor unless it were interesting in form or resemblance to some familiar object.

Appetite as shown by order of eating vegetables. Almost invariably the children ate their foods in rotation; in general when a food was tasted the whole serving was eaten before other foods were tasted. Cooked vegetables were usually eaten second or third with the bread and butter sandwich or the meat first. In the first group creamed turnips and in the second group creamed onions remained on the plate till last. In the groups fed unfamiliar vegetables, the order of tasting and finishing individual vegetables did not check in the two groups. Marked desire for a food seems to develop gradually.

Appetite for raw vegetables. In observation of the group of 10 children in their homes and again of the first groups in the nursery school the greater desire of children for raw vegetables was manifest. Nine common vegetables or vegetable mixtures were served raw to the first group and six to the second. Raw carrots and turnips cut in slender sticks were especially liked but cabbage shredded and served with dressing as a salad was received almost indifferently. After the first five-week period of feeding unfamiliar vegetables, those which had been most favorably received were fed during a second five-week period, making variations in texture, and this procedure was repeated with another group of children. Texture variations were made by serving the vegetable usually in four forms; raw sticks or large pieces, cooked sticks or large pieces, raw chopped and puréed cooked. Scoring variations in texture by tabulating verbal remarks showed no significant difference between the first three forms but the

puréed cooked were not eaten with relish. However, scoring by facial expression, an objective method, or by order of tasting and finishing the vegetable, the raw in large pieces was far in the lead if one accepts the assumption that young children eat first what they like best.

An experiment was carried out to test further this appetite of children for raw vegetables. During two five-week periods, two small groups, numbering seven and three children respectively, were allowed at three noon meals each week to choose between raw and cooked vegetables. In preparation of a vegetable, the edible portion was cut into uniform pieces, then half was crisped in ice water and the other half cooked until tender in just enough water to cover. Little plates with small bits of the raw and the cooked were placed on the table and the children were asked to taste both.

After the large plate with the rest of the food on it was put before the child he was allowed to choose the vegetable wanted. Out of 68 choices the raw was taken 55 times. Raw cabbage was taken 14 out of 15 times, raw carrots 18 in 22 times, raw cauliflower 7 in 12 times, raw celery 4 in 6 times and raw turnips 12 in 13 times. Whether due to more pleasing flavor or to crispness, raw vegetables were much better liked than cooked ones whether they were served in the form of sticks, cubes, large irregular pieces or were shredded or ground. In general, if they were cut so that they were readily picked up in the fingers they were eaten first, otherwise second or third among the foods served.

The principal objection to raw vegetables has been due to their fiber, a difficulty in masticating and an indication of indigestibility. The child seems to enjoy chewing crisp food. Perhaps we are passing through the period of caution with raw vegetables; many children are eating them with apparently great desire and without apparent harm, when care is taken to serve tender products only.

Summary

Records of the food of 42 preschool children show that they are eating a little more than two servings of vegetables per day in addition to potatoes. The variety has increased during the past twenty years to include most of the common vegetables.

An individual dietary study of six preschool children, with analysis of the food, showed that they receive 6 oz. of vegetables per day. This was one-seventh of the total weight of food as served and furnished about 10 per cent of the total energy and 17 per cent of the total iron. The total solids of mixed vegetables are rich in minerals.

It is important with preschool children to develop an appetite for vegetables so that an increasing quantity will be desired as the child grows. Feeding unfamiliar vegetables to 33 children certainly showed no refusal of new things introduced into the environment but rather the interest that young children have in new things and the strong appeal made by foods served in pieces of attractive shape. Repeated observations of 28 children showed that with the possible exception of strong-juiced vegetables, young children like vegetables to which they have become accustomed, especially if they are crisp in texture.

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THE VITAMIN B AND G REQUIREMENTS OF LACTATION*

By

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THE fact that an adequate quantity of vitamin B, old nomenclature, is in some way related to successful lactation has been observed for some time. Hartwell (1) in 1924 found that better milk production was possible in white rats if the amount of vitamin B in the diet was increased above the quantity necessary for growth. Guest, *et al* (2) in 1926 using cereal grains in the diet of white rats obtained results to show that the amount of vitamin B necessary for normal lactation is greater than that required for normal growth and that the amount of vitamin B required for reproduction is not much greater than that required for normal growth. The investigations of Sure (3) in 1927 showed that the young of mothers depleted of vitamin B developed paralysis, muscle chills, and screaming, running fits and that the symptoms were relieved by the administration of yeast. Macy and coworkers (4) in 1927 found that three to five times more vitamin B is necessary for lactation than for growth.

With the recognition of the two factors now believed to be a part of the original vitamin B, Evans and Burr (5) in 1927 reported that "the *additional* yeast needed for lactation is solely due to its addition to the antineuritic vitamin B and not to the growth-promoting vitamin B of the diet." Sure (6) in 1928 believed both factors to be necessary in added quantities for successful lactation. The present experiments have been carried on further to study the relative requirements of vitamins B and G for lactation. The data obtained are indicative of the fact that while both vitamins B and G must be present to obtain milk production, the lactation is usually more successful if vitamin G is fed in larger quantities than the amount which is given for growth.

EXPERIMENTAL PROCEDURE

Adult albino rats were used in these experiments. Each rat was placed in an individual, raised bottom cage. The food was placed in white por-

* The preliminary work of this investigation was submitted by Dorothy L. Hussemann in partial fulfillment for the degree of Master of Science in Home Economics in the Graduate School of the University of Illinois, 1929.

celain jars with screw tops, having an opening one and one-half inches in diameter. Supplements to the main diet were given in small, glass, castor cups. A few days previous to the birth of the young, the animals were transferred from the small cages to specially constructed cages, used in these experiments (Figure 1). No bedding was used until all of the young were born and then the rat was given shredded tissue paper for her nest. The cages, trays and food cups were changed weekly and the water cups, castor cups and bedding were changed daily. All of the apparatus was washed and sterilized with steam before being used again.

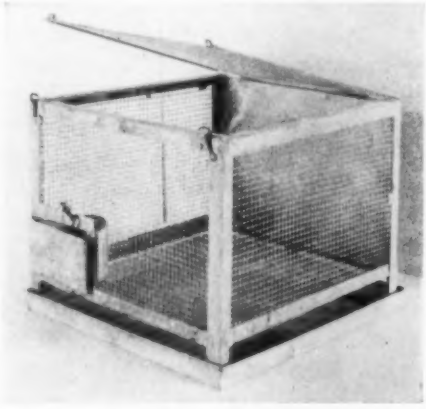

FIGURE 1.

The type of cage used in these experiments during the period of lactation. The animals were left in the small cages until just a few days before the young were born. At this time they were transferred to the large cage and allowed to remain there throughout the period of lactation.

The rats were weighed every three days until the birth of the young and then during the period of lactation were weighed every day. The litters, also, were weighed each day. The animals were mated in small cages, one male being used for each female. They were allowed to remain together for four days, at which time the male was removed. During the first experiments it was assumed that the female was pregnant and on the same day that the separation from the male occurred, she was transferred to the test diet. In the later experiments the female remained on the test diet throughout a preliminary rest period of three weeks in addition to the period of pregnancy and lactation. Four days after the birth of the young the litter was reduced to six (Evans and Burr, 5). Success in lactation was judged by

the growth of the young and their general nutritive condition. The period of lactation was considered to be twenty-one days in length.

The animals were allowed to eat *ad libitum*. The food intake was noted each time an animal was weighed or whenever a new supply of food was given. Distilled water was also given *ad libitum*. During the first experiments the female was placed on Diet A, called the "resting diet," for at least two weeks before mating. This was dispensed with, however, during the later experiments and the animals were given the test diet throughout the entire period. In addition to the "resting diet" Diets B, C, D, E, F, G and H were tested. The composition of the diets is given in Table I.

Observation will show the basal constituents of all of the diets to be the same, that is, they contain vitamin B-free casein[1] (except Diet A), dextrin or glucose,[2] Osborne-Mendel salt mixture, and various combinations of untreated and autoclaved yeast supplemented by cod-liver oil and wheat germ oil. The amount of casein in the diet was constant in all cases. It is approximately the same percentage as that used by Evans and Burr (5) in their studies on lactation. The amount of salt mixture incorporated into the diet is the same as that chosen by Chick and Roscoe (7) and Evans and Burr (5). Dextrin or glucose was a variable factor being increased or decreased according to the amount of yeast used. The dextrin was prepared by mixing cornstarch with cold water and then adding hot water. The mixture was placed in an oven at one hundred sixty-two degrees centigrade and allowed to cook until the mass in the pan was clear and opalescent, more water being added, if necessary. When the starch paste was clear the heat was reduced to one hundred and seven degrees centigrade and the oven door was opened slightly. In this manner the wet mass was allowed to dry. After being dried, the dextrin was ground to a fine powder and it was then ready for use as a source of carbohydrate in the diet. Because of the uncertainty as to the vitamin content of the dextrin, and the fact that in some growth experiments in our laboratory (10) glucose was shown to be free of vitamins B and G, glucose was used as a source of carbohydrate in the latter experiments. It was also found easier to use, requiring no preparation. Five drops of cod-liver oil were fed daily to each mature animal (Macy, 4). The wheat germ oil was made according to the cold acetone method of Sure (8). It was used to supply the vitamin E factor for which Evans and Burr (9) found the minimum protective dose to be 25 milligrams. This is approximately equivalent to three drops, so the latter amount was fed each day.

[1] Harris Laboratories, Tuckahoe, New York.

[2] Merck and Company, Rahway, New Jersey.

TABLE I

SHOWING COMPOSITION OF "RESTING DIET" AND OF TEST DIETS

RESTING DIET			
Diet A	Per cent		
Casein—commercial	30		
Salt mixture*	4		
Untreated yeast	5		
Dextrin	61		
TEST DIETS			
	First Experiments		Later Experiments
Diet B	Per cent		Per cent
Casein (Vitamin B-free)	30		30
Salt mixture*	4		4
Untreated yeast	15		20
Carbohydrates	51	dextrin	46 glucose
Diet C			
Casein (vitamin B-free)	30		
Salt mixture*	4		
Untreated yeast	5		
Autoclaved yeast	5		
Dextrin	56		
Diet D			
Casein (Vitamin B-free)	30		
Salt mixture*	4		
Untreated yeast	5		
Autoclaved yeast	10		
Dextrin	51		
Diet E		*Diet E*	
Casein (Vitamin B-free)	30		30
Salt mixture*	4		4
Untreated yeast	5		5
Autoclaved yeast	15		15
Carbohydrates	46	dextrin	46 glucose
Diet F		*Diet G*	
Casein (vitamin B-free)	30		30
Salt mixture*	4		4
Autoclaved yeast	15	untreated yeast	5
Dextrin	51		61 glucose
		Diet H	
		Diet G+3 drops of tikitiki daily	

* Osborne and Mendel.

In addition, in each case, five drops of cod-liver oil and three drops of wheat germ oil were given daily.

Tikitiki,[3] the dilute alcoholic extract of rice polishings, was used as a source of vitamin B. It has been shown by many workers to be potent in

[3] Bureau of Science, Manilla, Philippine Islands.

this factor. There is some indication, however (10), that tikitiki also contains vitamin G. The amount used in these experiments was the same as that used by Evans and Burr (5). An attempt was made to destroy the vitamin G fraction of yeast by irradiation following the technique of Hogan and Hunter (11) and then to use this product as a source of vitamin B in the diet. By testing the irradiated yeast on growth-experiment animals, however, it was found that there was practically no discernible destruction of vitamin G. This was later confirmed by Kennedy and Palmer (12) who found that only a very small quantity of vitamin G is destroyed by irradiation. Hence, in the experiments untreated yeast,[4] or untreated yeast and tikitiki were used to supply vitamin B in the diet. The source of vitamin G used throughout the experiment was autoclaved yeast. This was prepared by placing dried yeast in uncovered glass petri dishes to a depth of one-half inch. The yeast was not moistened and without further treatment was put in the autoclave. Here it was heated at one hundred twenty degrees centigrade with fifteen pounds of pressure for five hours. Then it was removed from the autoclave, dried, and ground to a powder. Frequent tests on growth animals showed this yeast to be a potent source of vitamin G, free from vitamin B.

During the first weeks of these experiments the yeast was given separately as a supplement to the diet. Such a procedure was unsuccessful as the animal often refused the yeast. The yeast was then incorporated into the diet in a definite percentage. Five per cent was the amount found by Evans and Burr (5) to be necessary for maintenance and growth and fifteen per cent was needed for lactation so these percentages were used in our control diets. In Diet B, however, the amount of yeast was raised to twenty per cent to test the effect of this higher level. The amounts and combinations in the other diets were chosen and set up arbitrarily.

Discussion of Results

Reference to Table II shows that only three of the eight litters born to mothers whose diet included 15 per cent yeast for vitamins B and G and dextrin for carbohydrate were successfully nourished during the period of lactation. Evans and Burr (5) have reported that "only when 15 per cent yeast is added to Diet 232 that normality in mortality and weaning weight is approached." Our results indicated that even more yeast might be necessary. In the later experiments, therefore, the yeast was increased to

[4] Northwestern Yeast Company, Chicago, Illinois.

TABLE II

SHOWING THE DIET, SOURCES OF VITAMINS B AND G, SUCCESS IN LACTATION, WEIGHT AND CONDITION OF YOUNG, AND CONDITION OF MOTHER AT THE END OF LACTATION

Rat number	Diet	Source of vitamins B and G in the diet	No. of young at the beginning of lactation period	No. of young at the end of lactation period	Average weight of young Gms.	Condition at the end of the Experiment	
						Young	Mother
1493	G	5 per cent yeast	6	6	26.6	Small, slightly oily	Normal
801	B (First experiments)	15 per cent yeast	6	6	37	Nice	Normal
949			6	6	38	Nice	Normal
M25			6	6	32	Nice	Normal
793			5	None			
806			10	None			
807			14	None			
948			13	None			
948			10	None			
7	B (Later experiments)	20 per cent yeast	6	6	28.3	Sticky, yellow, oily	Sticky, yellow
1397			5	4	37.5	Nice	Normal
5			6	6	34.3	Fur not cleaned, diarrhea	Yellow and oily
1503			6	6	50.0	Nice	Normal
1502			6	6	46.3	Nice	Normal
1721			6	5	36.6	Nice	Normal
1722			3	None			

TABLE II (continued)

Rat number	Diet	Source of vitamins B and G in the diet	No. of young at the beginning of lactation period	No. of young at the end of lactation period	Average weight of young	Condition at the end of the experiment	
					Gms.	Young	Mother
790	C	5 per cent untreated yeast and	10	None			
802		5 per cent autoclaved yeast	9	None			
951	D	5 per cent untreated yeast and	6	6	31	Fur thin	Normal
952		10 per cent autoclaved yeast.	6	6	26	Nice	Normal
M15	E (First experiments)	5 per cent untreated yeast and 15 perccent autoclaved yeast	6	2	16	Fur rough, gray paralysis and recovery	Normal
M18			6	5	34	Nice	Normal
949			6	6	34	Nice	Normal
1563	E (Later experiments)	5 per cent untreated yeast and 15 per cent autoclaved yeast	6	5	30	Nice, small	Normal
1562			6	6	38.8	Nice	Normal
1500			6	6	41.6	Nice	Mite sores
1498			6	6	38.6	Nice	Normal
1504			6	6	30.6	Nice	Normal
1727			5	None			
805	F	15 per cent autoclaved yeast	Mother became yellow and oily. Hemorrhages occurred at the time of parturition and the young were never born.				
806							
953							

TABLE II (continued)

Rat number	Diet	Source of vitamins B and G in the diet	No. of young at the beginning of lactation period	No. of young at the end of lactation period	Average weight of young	Condition at the end of the experiment	
					Gms.	Young	Mother
1570	H	5 per cent untreated yeast and 3 drops of tikitiki daily	6	6	38.3	Nice	Rough and slightly oily
1568			6	6	39.3	Nice	Normal
1569			6	6	38.0	Fur not clean	Abscess on mother's neck
1725			6	6	36.3	Nice	Normal
1724			10	None			
1459			1	None			

20 per cent and only one of seven litters failed. This would indicate that yeast at higher levels than 15 per cent is even more conducive to successful lactation. Evans and Burr (5) further found that yeast at a level of five per cent in the diet did not furnish enough vitamin B for lactation but this quantity was sufficient to maintain an adult rat in good health. Only one rat in our experiments was fed a diet containing five per cent yeast. It was found that she successfully nursed a litter of six for twenty-one days. The weaning weights, however, were low. The fact that this rat was able to successfully maintain the young may be due to a storage within her body of one or more factors from previous experimental work. Additions to the maintenance level of yeast of one or the other fractions of the vitamin B complex gave results indicating the relative importance of the two factors for successful lactation.

As has been stated, autoclaved yeast was used as a source of vitamin G. In Diet C five per cent autoclaved yeast was added to five per cent untreated yeast in the basal diet, the dextrin being decreased to this amount. Implantation occurred and the pregnancy was normal but when the young

were born very little if any milk was secreted and the young died within a week. Following the indication of so marked a deficiency in the diet, the amount of autoclaved yeast was doubled. At the level of 10 per cent autoclaved yeast and five per cent untreated yeast, two rats successfully nursed litters of six for twenty-one days but the weaning weights were low. A weight of 40 grams at the end of twenty-one days was the standard which had been adopted. A diet containing 15 per cent of autoclaved yeast with five per cent untreated yeast was then tested. The young obtained weighed from 16 to 38 grams at three weeks of age and were normal in appearance with the exception of one litter of two which showed a paralysis of the legs with subsequent recovery. On 15 per cent autoclaved yeast alone a successful litter was never born. At the normal time of parturition bleeding from the vagina occurred in the mother. An autopsy showed that the young had apparently developed normally at first but had become hemorrhagic.

Evans and Burr (5) have reported that "the additional yeast needed for lactation is solely due to its addition to the antineuritic vitamin B of the diet, for when tikitiki is given to lactating mothers without increased yeast dosage, we can also produce normal lactation." In the experiments here reported we have been able to repeat this work, four litters in six being successful on a diet which contained five per cent yeast and three drops of tikitiki. The weaning weights approached normal ranging from a weight of 36 to 39 grams at twenty-one days of age.

Hence, it would seem that if a source of either vitamin B or G is added to the maintenance level of yeast in the diet, more successful lactation occurs. The basal diets used for these animals were carefully controlled and tests showed these diets to be free of both vitamins B and G. That autoclaved yeast is a pure source of vitamin G we have shown in growth experiments. That tikitiki is a pure source of vitamin B has never been so definitely shown. Chart I demonstrates the results of growth experiments using tikitiki as the source of both vitamins B and G. It will be noted that almost normal growth was obtained when the larger amounts of tikitiki were used. This indicates that some vitamin G is present in tikitiki. The results obtained with tikitiki, therefore, are doubtful as to interpretation. It may be either vitamin B or vitamin G or even both of these vitamins which are functioning.

Chart II shows the growth curves of the young and mothers of typical animals on Diets B, E, H and G. There is very little difference in the condition of the mother and the young whether the source of the B and G vitamins is untreated yeast to 20 per cent of the diet or five per cent un-

treated yeast and 15 per cent autoclaved yeast. It would appear possible, therefore, to obtain successful lactation without increasing the intake of vitamin B over the maintenance level provided sufficient vitamin G is given. The young of mothers whose diet included five per cent untreated yeast and 15 per cent autoclaved yeast were in better general nutritive condition than those of mothers whose diet included five per cent untreated yeast and three drops of tikitiki.

CHART 1 - GROWTH CURVES OF ANIMALS ON A VITAMIN B AND G FREE BASAL DIET PLUS TIKITIKI

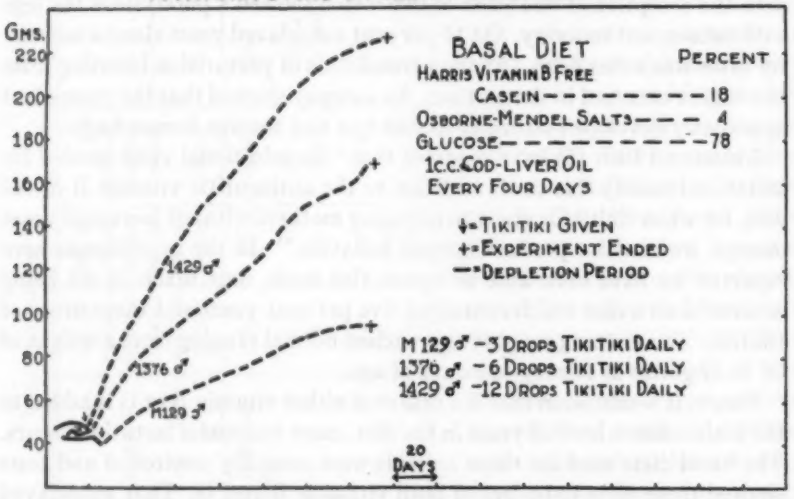

CHART 1.—Growth curves of animals on a vitamin B- and G-free basal diet plus tikitiki. The almost normal growth of animals receiving 12 drops tikitiki daily as the sole source of vitamins B and G indicates the presence of appreciable amounts of vitamin G in tikitiki.

Reference to Table III will show that in practically every case of successful lactation, there is a corresponding rise in food intake. All of the mothers who were able to suckle their young for twenty-one days ate comparable amounts of food during the period.

SUMMARY AND CONCLUSIONS

In this paper experiments are recorded which were devised to study the relationship of each factor of the vitamin B complex to lactation. Albino rats were used as experimental animals. Untreated yeast at a level of five per cent of the diet was used as a source of the vitamin B complex. This is

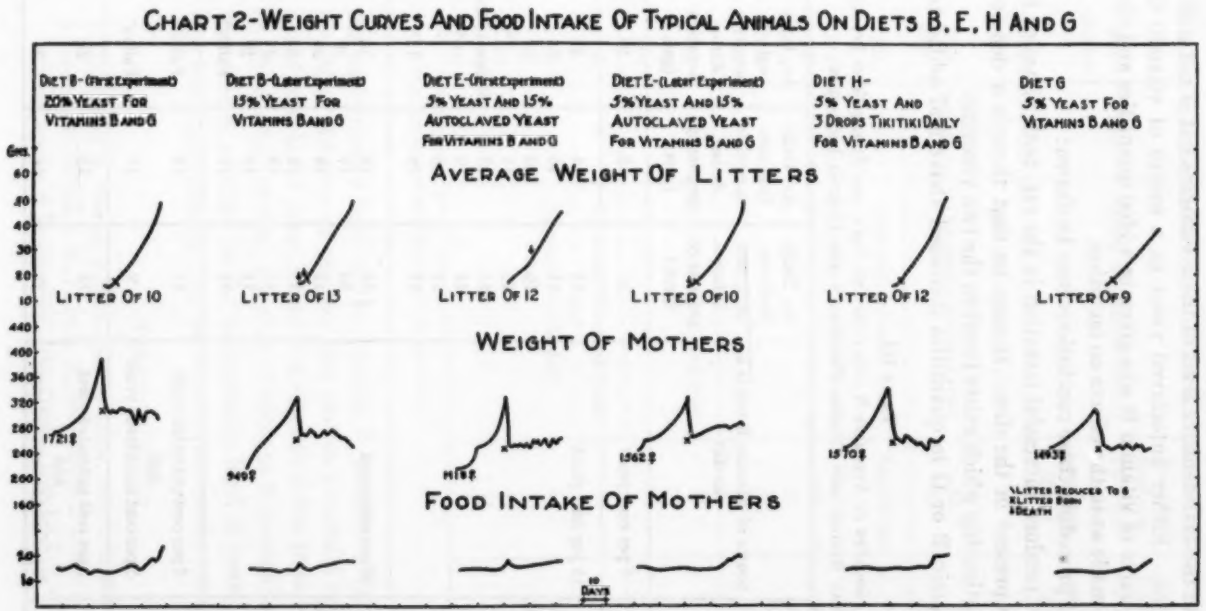
CHART 2-WEIGHT CURVES AND FOOD INTAKE OF TYPICAL ANIMALS ON DIETS B, E, H AND G
DIET B-(First Experiment) 20% YEAST FOR VITAMINS B AND G
DIET B-(Later Experiment) 15% YEAST FOR VITAMINS B AND G
DIET E-(First Experiment) 5% YEAST AND 15% AUTOCLAVED YEAST FOR VITAMINS B AND G
DIET E-(Later Experiment) 5% YEAST AND 15% AUTOCLAVED YEAST FOR VITAMINS B AND G
DIET H- 5% YEAST AND 3 DROPS TIKITIKI DAILY FOR VITAMINS B AND G
DIET G 5% YEAST FOR VITAMINS B AND G
AVERAGE WEIGHT OF LITTERS
Gms.
LITTER OF 10
LITTER OF 13
LITTER OF 12
LITTER OF 10
LITTER OF 12
LITTER OF 9
WEIGHT OF MOTHERS
1721 g
949 g
1562 g
1570 g
1693 g
1 LITTER REDUCED TO 6
1 LITTER BORN
1 DEATH
FOOD INTAKE OF MOTHERS
10 DAYS

CHART 2.—Weight curves and food intake of typical animals on diets B, E, H, and G. These diets include varying amounts of vitamins B and G. The food intake and weights of the mother rats are given during the periods of pregnancy and lactation. The growth of the young is recorded throughout the lactation period and in every case is indicative of the adequacy of the mother's diet for successful lactation. The almost normal growth of young of mother rats receiving five per cent yeast and 15 per cent autoclaved yeast for vitamins B and G in contrast to the slow growth of young of mother rats receiving five per cent yeast as the sole source of these vitamins indicates that it is possible to obtain successful lactation without increasing the intake of vitamin B over the maintenance level provided sufficient vitamin G is given.

Note: Diet B (First Experiment) above should read 15% yeast, and Diet B (Later Experiments) should read 20% yeast.

known to furnish the maintenance level of these vitamins but is not sufficient for lactation. Either autoclaved yeast as a source of vitamin G or tikitiki as a source of vitamin B was given in added quantities and observations were made as to the effects on lactation.

Following this procedure these conclusions may be drawn:

1. In order to produce successful lactation in the rat, both vitamins B and G must be present in the diet. It may be that there is a definite quantitative relationship which exists between the two vitamins.

2. Either vitamin B or G in quantities increased above and added to

TABLE III

SHOWING THE DIET, SOURCES OF VITAMINS B AND G IN THE DIET, AND DAILY FOOD INTAKE OF EACH RAT BEFORE AND DURING PREGNANCY AND DURING LACTATION

Rat number	Diet	Source of vitamins B and G in the diet	Av. Daily food consumption before pregnancy (gms.)	Av. Daily food consumption during pregnancy (gms.)	Av. Daily food consumption during lactation (gms.)
1493	G	5 per cent yeast	12	16	19
801	B (First experiments)	15 per cent yeast	15	16	30
949			11	16	21
M25			10	20	20
793			12	11	7*
806			13	13	Refused food
807			14	9	6*
948			11	16	7*
948			11	15	12*
7	B (Later experiments)	20 per cent yeast	14	19	26
1397			10	17	39
5			12	14	26
1503			15	21	32
1502			13	18	24
1721			11	19	19
1722			12	17	Failed*
790	C	5 per cent untreated yeast and	11	14	Failed*
802		5 per cent autoclaved yeast	9	17	Failed*
951	D	5 per cent untreated yeast and	13	14	22
952		10 per cent autoclaved yeast	12	14	23

TABLE III (*continued*)

Rat number	Diet	Source of vitamins B and G the diet	Av. daily food consumption before pregnancy (gms.)	Av. daily food consumption during pregnancy (gms.)	Av. daily food consumption during lactation (gms.)
949	E (First experiments)	5 per cent untreated yeast and 15 per cent autoclaved yeast	11	14	23
M15			12	22	16*
M18			12	17	27
1563	E (Later experiments)	5 per cent untreated yeast and 15 per cent autoclaved yeast	Record lost	17	21
1562			21	17	34
1500			15	20	25
1498			14	18	27
1504			15	14	26
1727			14	22	Failed*
801	F	15 per cent autoclaved yeast	15	9	Failed*
806			13	6	Failed*
953			12	4	Failed*
1570	H	5 per cent untreated yeast and 3 drops of tikitiki daily	14	21	29
1568			16	18	27
1569			15	20	26
1725			12	14	22
1724			13	14	Failed*
1459			13	18	Failed*

* These mothers were unsuccessful in lactation.

the maintenance level of the vitamin B complex yields more successful lactation. In view of the fact that just as successful results followed the use of autoclaved yeast, a source of vitamin G free from vitamin B, as were obtained when tikitiki, a source of vitamin B containing also vitamin G, was used in addition to the maintenance level of yeast, it seems possible that the added vitamin G is the more important for successful lactation.

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Editorial Review

A COMPARISON OF FEEDING STANDARDS FOR DAIRY COWS, WITH ESPECIAL REFERENCE TO ENERGY REQUIREMENTS*

OF supreme importance in relation to the nutrition of mankind is the efficient and economical feeding of the foster mother of the human family—a subject of unusual scientific interest and complication in that it involves the consideration of requirements for maintenance, gestation, and gain or loss of body substance, as well as for the production of milk of widely differing composition, nutritive cost and value.

In view of the facts that dairy cattle are ordinarily fed under carefully controlled conditions, and that large parts of the rations employed in milk production consist of expensive commercial feeding stuffs, it is both practicable and necessary to conduct the business in accord with the principles of science; and as a means to that end the knowledge of the physiology of nutrition, as it applies to this branch of animal production, has been systematized and simplified, and expressed in convenient tabular form, for the guidance of the milk producer.

The realization of the need for such aids to efficient milk production antedated the present understanding of the principles of nutrition by approximately two generations. With the advance of knowledge in this field, therefore, the prevailing feeding standards have changed from time to time; and inasmuch as evolution in this relation is still in process, the recent course of development of these guides to feeding practice is of present concern.

This paper considers the principles of derivation of the feeding standards which have been most used, or have been most influential in the molding of opinion. Inasmuch as the quantitative nutritive requirements of dairy cattle have not yet been definitely and finally established, the standards are compared and discussed without critical judgment of their quantitative correctness.

The earliest recorded suggestions relating to the scientific feeding of dairy cows seem to have come from Haubner (1) in about 1840, but the first definite feeding standard for milch cows was formulated in 1858 by

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Grouven (2), and was expressed in terms of the crude protein, fat, and carbohydrates of feeding stuffs as measured by chemical analysis.

The Digestible Nutrient System

Wolff, in 1864 (3), took the next long step in advance by expressing nutritive requirements of farm animals in terms of digestible crude protein, fat and carbohydrates; and the influence of this standard is still potent in the present day. Wolff's standard was published annually, without fundamental change, in Mentzel and Von Lengerke's Agricultural Calendar, until 1896. This standard was derived from the results of a large number of experiments by different observers at different times. It was intended to meet the requirements of a good, average dairy cow. It did not distinguish, however, between the maintenance requirement and the production requirement, and did not make any allowance for variations in the milk yield. For these reasons Wolff's standard was strongly criticized by Prof. Julius Kühn (4) of the Halle Experiment Station.

Kühn proposed a feeding standard which was somewhat more flexible than Wolff's since it made separate allowances for maintenance and for milk production, and took into consideration the quantity of milk produced. Kühn's standard was intended not to obtain the maximum yield of milk possible, but the maximum economic yield, taking into account the relative prices of the concentrated feeding stuffs and of dairy products. In Kühn's standard the amide compounds were classified together with the digestible carbohydrates instead of with digestible protein.

In 1897 Dr. C. Lehmann of the Berlin Agricultural High School modified Wolff's feeding standard in such ways as to meet Kühn's major criticisms. The result, the Wolff-Lehmann standard, was published annually, until 1906, in Mentzel and von Lengerke's Agricultural Calendar, and was used throughout the educated world. In this standard the kinds and quantities of nutrients specified were varied in accord with the quantity of milk, on terms as satisfactory as provided by the knowledge of the times.

In 1903 important advance was made by Haecker who as a result of many years of study at the Minnesota Station showed that the nutritive requirements of milk production vary not only with the quantity of milk but also with the quality, and especially with the fat content; and published a digestible nutrient standard (5) embracing this idea.

In 1912 Savage (6) published a modification of Haecker's standard, the principal change being an increase of about 20 per cent in protein requirements. Savage expressed his standard in terms of digestible crude protein and total digestible nutrients.

Morrison's (7) is the latest digestible nutrient standard, and is widely used. For details see the table on pages 158 and 159. The maintenance requirements are from Haecker. Two values are given for crude protein requirement for milk production—the lower being Haecker's and the higher Savage's; and two values are also given for total digestible nutrients, the higher being the average of Haecker's and Savage's values and the lower being the same reduced arbitrarily by 10 per cent.

Kellner's Starch Equivalent System

As a result of energy balance studies by Voit and Pettenkofer (8), Rubner (9), Zuntz (10), Meissl and collaborators (11) and Kellner (12), which developed an understanding of the mutual replacement values of nutrients as sources of energy, it became clear that the digestible nutrients of feeding stuffs do not accurately represent their true nutritive values; and to Kellner is due the credit for the first practical application of this modern scientific knowledge, by means of a feeding standard for cattle published in 1905 (13).

With mature oxen as experimental subjects Kellner determined the terms on which food protein, fat, and carbohydrate are converted into body fat; and with the useful residuum of energy from the metabolism of starch as a measure, he expressed the production values of feeding stuffs in terms of starch equivalents.

In the development of these measures Kellner added starch, straw pulp, sugar, wheat gluten and oil to basal maintenance rations, and by relating the body gains of energy, caused by the addition of these materials, to the energy of the digestible portions of the same, he determined the following body-fat equivalents (14):

1 Kgm. of digestible protein	2233	Cals.,	equivalent	to	235	gms.	body fat
1 Kgm. digestible starch and crude fiber	2356	"	"	"	248	"	"
1 Kgm. digestible cane sugar	1786	"	"	"	188	"	"
1 Kgm. digestible fat	4503–5681	"	"	"	474–598	"	"

Kellner chose the nutritive energy of 1 kgm. of starch as the unit of reference in the expression of production values. On the assumption that starch is 100 per cent digestible, the production value of 1 kgm. of starch was considered equal to 248 grams of fat, or 2356 Cals., which may be considered as the net energy value of starch for body increase. With this value of carbohydrates as a standard, or as unity, digestible protein has a starch value of 0.95,[1] (235÷248) and digestible fat has a starch value varying

[1] Kellner gave the fraction as 0.94 and so it has been widely quoted. Actually this factor is 0.9476.

from 1.91 to 2.41, depending on the source. The lower value for fat represents the fat of roots and coarse fodders; the higher value represents the fat of oil seeds and oil cakes. The fat of grains and cereal by-products has an intermediate starch value of 2.12.

After having thus established the "production values" of the digestible nutrients, when added in pure, finely-divided form, Kellner proceeded to test the applicability of the factors to ordinary feeding stuffs of cattle. This was done by respiration experiments carried out in exactly the same way as was done with the pure nutrients. A known quantity of the feeding stuff to be tested was added to a basal ration sufficient for maintenance; the digestibility of the added feeding stuff, as well as the quantities of protien and fat produced, were determined by difference; and the total gain of energy was compared with that computed from the digestible nutrients by the use of previously determined factors.

These comparisons showed that with oil meals the "production values" found by direct experimentation agreed closely with the values computed, by the use of the factors, from the quantities of protein, carbohydrate, and fat actually digested. Very different results, however, were obtained with hays and other roughages. With these feeding stuffs the calculated increase in fat did not agree with the observed, the latter being considerably smaller in all cases. Wheat straw showed a difference of 70–80 per cent, oat and barley straws and various varieties of hay showed differences of 30–40 per cent. Kellner accounted for this apparent deficit as resulting from increased work of mastication and digestion, and increased energy loss through fermentation, both being due to the high crude fiber contents of these feeds. He computed that 100 grams of crude fiber diminished the fat production by 14.3 grams, which was equivalent to 58 grams of starch. This correction was considered applicable to hays. For the chaff of various cereals Kellner estimated that the diminution was equivalent to only 29 grams of starch. For green plants, containing 16 per cent or more of crude fiber, the same correction was considered necessary as for dry forage; and if the amount of crude fiber was only 4 per cent or less the correction was the same as for chaff, while between these limits a sliding scale was arbitrarily used. These corrections, based on crude fiber, were applied only in the computation of the production values of roughages from their digestible nutrients.

In the case of concentrated feeds, however, the crude fiber content was found not to be a satisfactory basis for measuring the differences between the observed production values and those computed from the digestible

nutrients by means of the starch equivalents, thus showing that the crude fiber was not the only factor accountable for this difference. In order to account for this discrepancy, therefore, Kellner determined, for a number of concentrates, the observed production value as a per cent of the computed, and considered this percentage as a correction which must be applied to the value computed from the digestible nutrients in order to obtain the true production value. This correction was designated by Kellner as a "Wertigkeit," or evaluating factor. Wheat bran, for example, was found to have a "Wertigkeit" of 77.3 per cent. This means that the computed starch equivalent of wheat bran must be multiplied by 0.773 in order to obtain its true production value.

According to Kellner's system of computing the energy values of feeds it is therefore necessary to compute the starch equivalent from the digestible nutrients and then to correct these values by the employment of special evaluating factors characteristic of the type or class of feeding stuff. In case of roughages a minus correction is made, based on the total crude fiber content of the feed, while in the case of concentrates the percentage correction may be plus or minus. Such percentage corrections were directly determined by Kellner for only a relatively small number (twelve) of concentrates. For the majority of feeding stuffs they were assumed.

Kellner's production values, therefore, are in a sense net energy values, but the method of computing them is highly conventional.

As a basis for the utilization of these starch values in the formulation of a feeding standard for milk production Kellner (15) compared the economy of utilization of feed energy for fattening and for milk production, and found that the latter is decidedly the more efficient function. Kellner's results as recomputed by Armsby (16) show that the utilization of the metabolizable energy for milk production, for three cows, was 68.4 per cent, 72.8 per cent, and 66.9 per cent, respectively, while the corresponding values for fattening were 48.0 per cent, 46.4 per cent, and 43.8 per cent. This finding was corroborated by later investigators, as will be brought out hereinafter.

In these experiments Kellner obtained a good agreement between the computed starch values of the food available for milk production and the starch values computed from the milk constituents, on the basis of the assumption that milk protein and milk sugar are produced from the digestible protein and digestible carbohydrate, respectively, of the food, without any expenditure of energy, and that milk fat is produced from carbo-

hydrate at the same energy expense as is body fat. In accord with this assumption Kellner determined that 1 kgm. of milk fat has a starch value of 3.9 kgm; that 1 kgm. of milk sugar has a starch value of 1.05 kgm; and that 1 kgm. of milk protein has a starch value of 0.94 kgm.

On the basis of these factors, and on the assumption of an average composition of milk of 3.2 per cent fat, 4.6 per cent lactose and 3.3 per cent protein, Kellner computed that for the production of each 10 kgm. of milk 2.0 kgm. of starch values are required. He also estimated that for milk of a high fat content as much as 2.7 kgm. starch value may be required per 10 kgm. of milk.

Kellner estimated the maintenance requirement of the dairy cow on the basis of his results with oxen. For the latter he found that 2 kgm. of starch value represented the average daily maintenance requirement for 500 kgm. of live weight. For cows he considered it necessary to allow 0.5 kgm. additional starch value as a factor of safety, thus making in all 2.5 kgm. of starch value per 500 kgm. live weight.

Kellner expressed the protein requirement in terms of digestible true protein. His estimate for maintenance was 0.3 kgm. per 500 kgm. live weight, and for milk production 0.55–0.65 kgm. of digestible true protein per 10 kgm. of milk.

Kellner utilized these conclusions in the formulation of his latest feeding standard for dairy cows, stating the requirements for maintenance and for milk production together, per 1000 kgm. live weight, as follows:

Milk yield per day per 500 kgm. live weight kgm.	Starch value kgm.	Digestible true protein kgm.
5	7.8–8.3	1.0–1.3
10	9.8–11.2	1.6–1.9
15	11.8–13.9	2.2–2.5
20	13.9–16.6	2.8–3.2

It is apparent that in this standard of Kellner the allowances for milk production are on the basis of his estimates given above, *viz.* 2.0 to 2.7 kgm. starch value and 0.55 to 0.65 kgm. digestible protein per 10 kgm. of milk. When these allowances for milk are deducted from the total allowances, the quantities left for maintenance are 5.8 kgm. starch value and 0.5 to 0.7 kgm. digestible true protein per 1000 kgm. live weight.

The protein quota is in accord with Kellner's estimate as given above, *viz.* 0.3 kgm. per 500 kgm. live weight, but the starch value 5.8 kgm. per 1000 kgm. live weight is appreciably above his estimate of 2.5 kgm. per

500 kgm. live weight, which already includes a factor of safety of 0.5 kgm. The basis for this difference is not revealed in Kellner's writings.

Inasmuch as in Kellner's standard the requirements for maintenance and for milk production are stated together and no separate allowances are made for the different grades of milk, as to fat content, this standard is not readily usable for the computation of rations of individual animals.

Armsby's Net Energy System

Armsby (17), with the same fundamental conceptions as Kellner's, first expressed all of the factors of the process of utilization of food by cattle in terms of calories, and developed the point of view which has become known as the net-energy system. In 1917 he (16) formulated a feeding standard for the milch cow expressed in terms of true protein and net energy.

There is a close similarity between Armsby's net-energy values and Kellner's production values of feeding stuffs. Both were based on complete energy balances, but Armsby's system is simpler and more direct, and less likely to be misunderstood, since he measured energy values in calories, while Kellner measured the same in terms of matter.

According to Armsby the entire expenditure of energy due to the consumption of a feeding stuff is determined from a comparison of the heat production on a basal ration and the heat production on the same ration plus the feed under investigation. This is called the heat increment of the feed. The net energy of the feed is then obtained by subtracting the heat increment from the metabolizable energy, the latter representing the gross energy minus the energy of the feces, urine and methane.

Roughages are found to contain relatively less net energy than do concentrated feeds, not because they contain much crude fiber which causes much mechanical work, and not necessarily because their heat increment values are higher than in the case of concentrates, but because the heat increment value is subtracted from a much smaller amount of metabolizable energy.

Since the direct determination of net-energy values of feeding stuffs is necessarily slow and expensive Armsby and Fries (18) developed, from respiration calorimetric experiments, the following factors for computing metabolizable energy from the digestible organic matter of feeds, these factors to be employed in deriving provisional net-energy values for temporary purposes:

For roughage.....1.588 Therms per pound of digested organic matter.
For grains and similar feeds
With less than 5% digestible fat.....1.769 Therms per pound of digested organic matter.

With more than 5% digestible fat.....1.814 Therms per pound of digested organic matter.
For oil meals.....1.996–2.177 Therms per pound of digested organic matter.

In order to compute the net-energy value of a feeding stuff, according to Armsby, it is necessary to multiply the pounds of digestible organic matter by one of the factors given above and then to subtract the heat increment value of the particular feed, or of a similar feed.

Armsby's net-energy values apply, directly, to body increase. In order to be able to employ them in the expression of nutritive requirements for milk production, therefore, he made special provisions.

From a consideration of experimental data of Kellner (15), of Jordan (19), of Haecker (5), and of Eckles (20), Armsby was convinced that the utilization of the food energy for milk production is considerably more efficient than for fattening; and in an attempt to explain this difference he considered it probable that "the conversion of digestible proteins into milk proteins, or of digestible carbohydrate into milk sugar, may involve a comparatively small expenditure of energy as compared with the synthesis of fat from carbohydrates or protein." As a working hypothesis, therefore, he assumed that the digestible protein and carbohydrate of the feed may be converted into the corresponding compounds of milk without energy loss, and that the expenditure of energy in the production of milk fat from carbohydrates is the same as that observed by Kellner for the production of body fat. On the basis of this hypothesis, and Haecker's analyses (5) of milk of various grades as to fat content, Armsby (16) computed the amount of net energy for fattening which would be required for the production of each of these qualities of milk.

The values thus computed were increased by 5 per cent in recognition of the fact, shown by Eckles (21), that the digestibility of rations by well-fed cows is on an average about 5 per cent lower than as indicated by the average digestion coefficients given by Henry and Morrison (22), on which the computations of net energy values were based.

In formulating his feeding standard for the dairy cow Armsby stated separately the requirements for maintenance and the requirements for milk production. For energy maintenance he adopted as the standard 6.0 Therms of net-energy for a 1000-lb. cow. This figure was derived from a number of respiration calorimetric experiments, including both submaintenance and supermaintenance feeding, with steers, by a method involving the assumption that the heat production is a rectilinear function of the food consumption. Armsby and Fries (23) regarded the net-energy values of a food for maintenance and for body increase to be essentially alike. From the

results of work which Armsby planned but which was conducted after his death (24), it is clear that the utilization of food energy for maintenance is distinctly more efficient than for body increase. This finding rests upon the fact (25) that the directly observed fasting katabolism of cattle is a considerably higher value than is that computed, according to Armsby's method, from a comparison of the heat production on two different planes of feeding.

Armsby expressed the protein requirements for the dairy cow in terms of digestible true protein. For maintenance his estimate, based on his own experiments and on results of other observers, was 0.5 pound digestible true protein per 1000 pounds live weight. For milk production Armsby's allowance of digestible true protein was 1.6 times the milk protein in accord with Haecker's analysis. The details of Armsby's feeding standard for the dairy cow are given in the table on pages 158 and 159.

Møllgaard's Production Equivalent System

Prominent among the later studies by intensive methods are those of Møllgaard (26) who made many energy balance determinations with milch cows, and evolved a feeding standard embracing original ideas but based in part on Kellner's and Armsby's systems.

Møllgaard recognized the impossibility of determining net-energy values of feeds for milk production by the "difference" method, and followed Armsby in expressing the energy requirements for milk production in terms of net-energy for fattening, but computed his net energy values of feeding stuffs on the basis of Kellner's starch values.

The work of Kellner and Armsby suggested to Møllgaard that the fat content of the milk is an important factor in determining the net energy requirement for milk production. He therefore proposed, in 1923 (27), to provide for milks of different fat content by expressing the requirements of net-energy in accord with the milk energy—basing this procedure on his finding that the energy of milk is a linear function of its fat content—from which it follows that the former can be computed from the latter by the use of a simple formula. This formula for the Red Danish cow is $y = 280.6 + 115\,x$, and for the Jersey cow, $y = 363 + 101\,x$, in which y represents milk energy and x represents per cent of fat in the milk.

Møllgaard designated 1000 Cals. of milk energy as a milk unit, and the quantity of net energy (for fattening) required to produce such a milk unit he designated as a production equivalent; also, as a result of his experiments, he concluded that the production equivalents of a ration are affec-

ted by the protein content of the same. He therefore expressed the relation of the digestible true protein of the ration to the total digestible nutrients, in terms of net-energy, by computing the net-energy of the protein, by the use of Kellner's factor, which is 2223[3] Cals. per kgm. of digestible true protein, and the net energy of the total digestible nutrients also according to Kellner's procedure. This ratio of the true protein net energy to the total net energy of the food he calls the quotient of production (K). This quotient of production is comparable, in a general way, with the nutritive ratio with which all students of animal feeding are familiar, but has not exactly the same significance, since the nutritive ratio is a proportion of digestible crude protein (while Møllgaard's measure is based on true protein) to digestible non-nitrogenous starch equivalent (while Møllgaard's measure is based on total digestible nutrients).

Møllgaard (26) made a study of the influence of the quotient of production on the production equivalent, and arrived at the conclusion that the minimum production equivalent *i.e.*, the optimum utilization of food energy for milk production, is obtained when the quotient of production of the food available for milk production, *i.e.*, in excess of maintenance, is between 0.14 and 0.23.

From ten complete balances of matter and energy with milch cows—the quotients of production in the available food being kept within the range given above—Møllgaard computed that after allowing sufficient net energy for maintenance, the remaining net energy of the ration, in relation to the milk energy, gave production equivalents ranging from 794 Cals. to 875 Cals., with an average value of 837 Cals.

Møllgaard (26) also made a study of the influence of the percentages of fat in the milk on the production equivalents, and observed that the latter vary conversely as the fat content. He regards the differences as small, however, for practical purposes, and recommends the average production equivalent of 837 Cals. as the standard, and an average quotient of production (K) of 0.2.

Møllgaard's standard for the energy maintenance of the dairy cow is based on the following conceptions:

He accepts the view that net energy values for maintenance are different from those for fattening, and that the maintenance requirement of net energy, as computed by the "difference method," expresses correctly the energy requirement in terms of net energy for fattening,—provided that

[3] This value is as recomputed by Møllgaard (26). The value given by Kellner (14) is 224 Cals. per 100 grams of digestible true protein.

the rations compared are both above maintenance, or that one is at maintenance and the other above; and also provided that the protein requirements are satisfied in both.

Møllgaard compared determinations of net energy for maintenance as obtained in experiments of the Pennsylvania Institute of Animal Nutrition, involving supermaintenance rations, with similar values derived from submaintenance rations, and found that the average of ten values derived from supermaintenance rations (four of which being values for cows and six for steers) was 1024 Cals. per square meter of body surface; while the average of 14 values for steers, derived from submaintenance rations, was 1240 Cals. per square meter. On the basis of this comparison he concluded that the relationship between net energy values for fattening and net energy values for maintenance is $1024/1240 = 0.826$. Møllgaard then multiplied by the factor 0.826 the above-mentioned fourteen values of Armsby and Fries, for maintenance, computed by the difference method and involving submaintenance feeding, in order to convert them to terms of net energy values for fattening; and determined the average of all 24 determinations to be 5046 Cals. per 1000 lb. live weight. He also applied the same factor to values for the directly determined fasting katabolism of steers, as observed by Benedict and Ritzman (28), and by Forbes and associates (25), and found that an average of eleven determinations thus "corrected" was 5846 Cals. per 1000 lb. live weight. The average of 5046 Cals. and 5846 Cals., that is, 5446 Cals., per 1000 lb. live weight, is Møllgaard's standard for the maintenance requirement of the dairy cow in terms of net energy for fattening.

This procedure of Møllgaard, in arriving at his standard for maintenance, is subject to serious criticism. It was shown by Forbes and associates (29, 30), in their study of the influence of the plane of nutrition on the energy metabolism of cattle, that the heat production at points between fasting and energy equilibrium is expressed by a distinct curve, from which it follows that net energy values of rations below maintenance must differ considerably in accord with the quantity eaten, the efficiency of utilization of food energy diminishing rapidly with increase in food from the status of fast to the point of energy equilibrium. In the light of these studies it seems unjustifiable to apply the factor 0.826, derived by the procedure stated, to values representing the directly determined fasting katabolism for the purpose of obtaining the maintenance requirement in terms of net energy for fattening. The fact that the application of the factor 0.826 to the fasting data yields results distinctly higher than those obtained by ap-

plying this factor to the maintenance values computed by the "difference procedure" from submaintenance rations is in itself proof that the factor 0.826 is not applicable to the fasting data. In other studies with cows Forbes and associates (24) have compared the efficiency of utilization of food energy for body increase with the utilization of the same for maintenance—the latter involving the use of the directly determined fasting metabolism,—and found an average relationship of 1 for maintenance to 0.761 for body increase. Obviously, it is not possible to have one conversion factor applicable to different planes of nutrition.

Møllgaard also believes that the net energy of the true protein quota for maintenance, and the total net energy quota for the same, should be in the proportion of 1 to 10.

Møllgaard's expression of protein requirements for maintenance and for milk production as net energy of digestible true protein, as has been explained, renders this system somewhat complicated and difficult to use.

On several accounts it is most convenient, and otherwise desirable, that the allowance of protein should be made separately from the quota for energy production. Cows adapt themselves with no apparent disadvantage to wide differences in protein intake; and the allowance of protein may properly be modified in accord with its biological value and in accord with the relative cost of feeding stuffs. It is therefore questionable whether there is an advantage in Møllgaard's method of expression, in which energy-producing nutriment and protein are assigned in combination, and in which the quantity of protein is obscured, for most persons, by the method and terms of its expression; also, as Halnan (31) pointed out, it is technically incorrect to assign energy and protein for maintenance on the same basis, as they are by Møllgaard's standard, since the former is computed in relation to body surface while the latter should be computed in relation to body weight.

Hansson's Food Unit System

The early Scandinavian systems of estimating comparative values of feeds for milk production were based on feeding trials without chemical control; thus Fjord (32), in 1887, initiated a "food unit" system in which the measure was the nutritive value of 1 kgm. of barley; and in Denmark a system came into vogue in which the measure was the nutritive value of 0.5 kgm. of mixed oats and barley (1:1); but in 1915 the Danish, Norwegian and Swedish control unions agreed upon 1 kgm. of barley as the standard unit.

The development of a scientific feeding standard for dairy cows, based on the food unit system, is due to Nils Hansson. As early as 1902 Hansson (33) estimated from results of practical experiments in Sweden that, for each 3 kgm. of average milk produced, 1 food unit in excess of the maintenance requirement is required. At a later date (34) he obtained the same average from control experiments involving over 110,000 cows, the average percentage of fat in the milk being close to 3.5. From the composition of milk containing 3.5 per cent fat Hansson calculated that 1 kgm. of milk contains, as an average, 700 Cals. Hence 1 food unit is equivalent to 3×700, or 2100 Cals. This value is considered by Hansson as representing the net energy value of a Swedish food unit for milk production; and the normal digestible protein content of a food unit for milk production is considered to be 135–150 grams.

From a comparison of Kellner's tables of starch values and his own tables of food units Hansson (34) computed that 1 food unit is equal to 0.7 kgm. of starch value. Since 1 kgm. starch value is equivalent to 2356 Cals. net energy for fattening, 1 food unit is computed to be equivalent to 2356×0.7, or 1650 Cals., of net energy for fattening. The difference between 1650 Cals., for fattening, and 2100 Cals., for milk production, computed as above, is explained by Hansson in the following manner:

According to Kellner 1 kgm. of digestible protein has a fattening value equal to 235 grams of fat, or $235 \times 9.5 = 2233$ Cals. net energy,—indicating that the utilization of the energy of the protein is 39.1 per cent ($2.233 \div 5.71$). Hansson found in his experiments with cows that about 75 per cent of the digestible protein is recovered in the milk—which is 36 per cent greater than the utilization of the energy of the same for body increase. Hansson further estimated that in 1 food unit, containing 135 grams digestible true protein, the difference of 36 per cent in the utilization of protein, is equivalent to 278 Cals. ($5.71 \times 135 \times 0.36$). Since 278 Cals. do not fully account for the difference between 1650 and 2100 Cals. (that is, the difference between the net energy of a food unit for fattening and for milk protion), Hansson ascribed the remaining difference to a greater utilization of the carbohydrates in milk production.

The above considerations led Hansson to assume that in the process of milk production the percentage utilization of protein is the same as that of carbohydrates, and that the starch equivalent of protein for milk production is therefore determined by the relation of the total energy of 1 gram of digestible protein (5.71 Cals.) to the energy of 1 gram of carbohydrates (4.0 Cals.), that is, it is equal to 5.71/4.0 or 1.43. On this basis

Hansson modified Kellner's computation of starch values by substituting the factor 1.43 for Kellner's factor 0.94 for protein, and leaving the factors for carbohydrates and for fats unaltered. The starch value of a food unit thus computed by the use of the new factor for protein is designated by Hansson as milk production value. The computation of the final milk production value involves also the use of a percentage correction (Wertigkeit) as does the computation of the starch values according to Kellner. In accord with this method Hansson computed the milk production values of Swedish feeding stuffs.

From a comparison of the computed milk production value with the Swedish food units Hansson estimated that, as an average, one food unit corresponds to 0.75 kgm. milk production value. Since 1 food unit is computed to be equivalent to 2100 Cals. net energy for milk production, 1 kgm. milk production value corresponds, according to this computation, to 2800 Cals. net energy for milk production.

Hansson's system of calculating values of feeds for milk production in the above manner was criticized by Fingerling (35) and by Møllgaard (36) mainly on the ground that the energy of protein is assumed to be completely metabolizable, and is defended by Kleiber (37) as being in agreement with results of practice.

The main substance of Hansson's feeding standard for dairy cows is given in the table on pages 158 and 159.

Forbes' and Kriss's Method of Derivation of a Feeding Standard for Dairy Cows

The most recent contribution to this problem has come from the Pennsylvania Institute of Animal Nutrition. In the light of studies initiated by Armsby, and continued after his death,—embracing complete energy balances with milch cows, and extended studies of fundamental principles underlying net-energy determinations—Forbes and Kriss (38) have devised a new method of application of the net-energy conception, to derive the energy requirements of milk production, optionally, in terms of metabolizable energy or total digestible nutrients, in a manner serving to avoid certain of the serious difficulties involved in direct net-energy determinations.

The studies of Forbes and associates (24, 25, 29, 30) on the energy metabolism of cattle have revealed numerous unsolved problems involved in the determination of net-energy values of individual feeding stuffs; and not only the earlier results of Armsby and Fries (23), but their own later

determinations (30), are characterized by such variability as emphasizes the need for further investigation of the practical application of the net energy conception.

Also these studies have removed all possible doubt as to the fact that the efficiency of utilization of feeding stuffs for maintenance differs from the same for body increase, and that the efficiency of utilization for body increase differs from the same for milk production.

Further, these studies have shown that no entirely satisfactory methods have as yet been found for determining net-energy values of individual feeding stuffs for maintenance, for body increase, or for milk production.

In the light of these observations Forbes and Kriss (38) have arrived at the conclusion that the net energy conception applies much more satisfactorily in the evaluation of approximately complete rations than to the determination of the energy values of individual feeding stuffs; have considered the problem of the method of application of this principle to the derivation of feeding standards; and by the use of three energy relationships have devised a new method and principle for utilizing the results of complete energy balances in the formulation of guides in feeding practice.

These three energy relationships are (1) the utilization of metabolizable energy for milk production, (2) the utilization of metabolizable energy for body increase, and (3) the relation between the metabolizable energy of approximately complete rations and the "total digestible nutrients" (the sum of the digestible crude protein, the digestible carbohydrate, and the digestible ether extract multiplied by 2.25) of the same rations.

Regarding the above-mentioned energy relationships the following facts were established:

1. The utilization of the metabolizable energy of normal, mixed rations for the purpose of milk production, as determined in three complete energy balances by Kellner, and in eleven such at the Pennsylvania Institute of Animal Nutrition, was found to agree very well—the average of the 14 determinations being 69.3 per cent.

2. The utilization of the metabolizable energy of seven, normal, mixed rations for the body increase of cattle (steers and cows) was found in 32 determinations at the Pennsylvania Institute of Animal Nutrition, to average 58.4 per cent, with a coefficient of variation of 5.83 ± 0.51 per cent; and in seven such determinations with cows alone, on rations all of the same character, the utilization averaged 57.5 per cent.

3. The relation of the metabolizable energy of normal mixed rations to the total digestible nutrients of the same, was found, in 77 respiration ca-

lorimetric experiments at the Pennsylvania Institute of Animal Nutrition to be remarkably close. The average metabolizable energy per pound of total digestible nutrients was 1.616 Therms, with a coefficient of variation of 3.57 ± 0.27 per cent.

On the basis of the foregoing observations Forbes and Kriss have proposed to determine and to express the energy requirements of the dairy cow in the following manner:

The gross energy of the milk is considered to be the measure of the net-energy requirement of milk production, and is related to the feed required to produce it, in terms of metabolizable energy, as a percentage thereof. In other words, the metabolizable energy requirement for milk production may be obtained by dividing the gross energy of the milk (which may be estimated from the fat content) by the established average percentage utilization of metabolizable energy for milk production of normal mixed rations (69.3 per cent). For convenience in the computation of rations the metabolizable energy requirements for milk production are then computed to equivalent quantities of total digestible nutrients by the use of the experimentally established factor representing this relation in normal mixed rations (1.616 Therms = 1 pound total digestible nutrients).

The maintenance requirement of the animal is determined, in terms of metabolizable energy, as the total heat production of the animal, on a plane of energy equilibrium, on a normal mixed ration. This is accomplished in a single, simple, direct experiment, with a proper accounting for such small gain or loss of energy as occurs. The average of seven such direct determinations, with four cows, was found to be 8.487 Therms per 1000 lbs. live weight. For convenience in the computation of rations the metabolizable energy requirement for maintenance, like the metabolizable energy requirement for milk production, was computed to an equivalent quantity of total digestible nutrients.

The authors of this system state that inasmuch as the maintenance value was determined in a respiration calorimeter it will be revised, presumably by increase, as more data are available, and as a definite basis shall be found for rendering it applicable to conditions of practice.

In their recently formulated feeding standard Forbes and Kriss express the protein requirement in terms of digestible crude protein. The maintenance quota is 0.6 pound per 1000 pounds live weight, while for milk production an allowance is made of 1.25 to 1.75 times the estimated protein of the milk, this latitude being indicated in recognition of various conditions of practice which are enumerated. The protein requirement for milk pro-

duction is, in reality, a phenomenally elastic value. It varies between a minimum of 1.0 times the milk protein, and, according to Perkins,[3] an optimum of probably more than 3.0 and less than 6.0 times the same. In practice the quantity of protein given is much influenced by economic considerations.

The tentative standard of Forbes and Kriss, presented to illustrate this method, is included in the table on pages 158 and 159.

A fundamental difference between this system of Forbes and Kriss and those of Armsby and Møllgaard is that while in the latter the net-energy requirements for milk production are converted by the use of experimentally determined factors to net-energy equivalents for fattening, in the system of Forbes and Kriss the net-energy requirements for milk production are converted by the use of experimentally determined factors to the equivalents of the same in terms of total digestible nutrients.

The net-energy and the starch-equivalent values of feeding stuffs are theoretically much more significant than are digestible nutrients, but, on the basis of present knowledge, digestible nutrients can be much more accurately determined than can net-energy and starch-equivalent values; and in the use of the digestible nutrient values, by the method of Forbes and Kriss, the digestible nutrients are related to the milk energy by a computation which adequately recognizes the energy losses between digestible nutrients and metabolizable energy, and between metabolizable energy and net energy. The system of Forbes and Kriss has the further advantages of being the simplest system; of employing more satisfactory measures of protein values and of energy requirements for maintenance; and of expressing nutritive requirements in measures with which all students of animal feeding are acquainted.

Conversion Factors for Measures of Nutritive Value

The quantitative relations between the feeding standards which have been discussed may be determined by means of the following conversion factors:

1 milk unit is equivalent to 1 Therm milk energy or 0.837 Therm net energy for fattening. (Møllgaard)

1 Kgm. digestible true protein is equivalent to 2.233 Therms net energy for fattening. (Kellner, Møllgaard)

1 Kgm. starch value is equivalent to 2.356 Therms net energy for fattening. (Kellner)

1 food unit is equivalent to 1.650 Therms net energy for fattening. (Hansson)

1 food unit is equivalent to 2.100 Therms milk energy. (Hansson)

[3] Personal letter to E. B. Forbes, of the date of January 23, 1931.

MAINTENANCE REQUIREMENTS, PER 1000 POUNDS LIVE WEIGHT PER DAY

Author	Energy			Protein	
	In original terms	Net energy (for fattening)	Total digestible nutrients	Digestible true protein	Digestible crude protein
		Therms	Pounds	Pounds	Pounds
Kellner (1912)	2.631 kgm. starch value*	6.200	6.673	0.5–0.7*	
Armsby (1917)	6.0 Therms net energy	6.000	6.456	0.5	0.6**
Møllgaard (1929)	5.446 Therms net energy	5.446	5.860	0.54	
Nils Hansson (1926)	3.175 food units	5.239	5.638	0.5	
Morrison (1923)	7.925 pounds total digestible nutrients	7.362	7.925		0.7
Forbes and Kriss (1931)	5.260 pounds total digestible nutrients	4.888	5.260		0.6

REQUIREMENTS FOR MILK PRODUCTION, PER POUND OF MILK

Author	Per cent fat in milk	Energy			Protein	
		In original terms	Net energy (for fattening)	Total digestible nutrients	Digestible true protein	Digestible crude protein
			Therms	Pounds	Pounds	Pounds
Kellner (1912)	all grades	0.091–0.122 kgm. starch value*	0.214–0.287	0.230–0.310	0.055–0.65*	
Armsby (1917)	3.0	0.214 Therms net energy	0.214	0.230	0.043	
	4.0	0.265 " " "	0.265	0.285	0.049	
	5.0	0.315 " " "	0.315	0.339	0.055	
	6.0	0.361 " " "	0.361	0.388	0.061	
	7.0	0.408 " " "	0.408	0.439	0.068	

REQUIREMENTS FOR MILK PRODUCTION, PER POUND OF MILK (*Cont.*)

Author	Per cent fat in milk	Energy			Protein	
		In original terms	Net energy (for fattening)	Total digestible nutrients	Digestible true protein	Digestible crude protein
			Therms	Pounds	Pounds	Pounds
Møllgaard (1929)	3.0	0.281 milk units	0.235	0.252	0.046	
	4.0	0.336 " "	0.281	0.302	0.056	
	5.0	0.390 " "	0.326	0.351	0.064	
	6.0	0.440 " "	0.368	0.396	0.073	
	7.0	0.485 " "	0.406	0.437	0.080	
Nils Hansson (1926)	3.0	0.15 food units	0.248	0.267	0.040	
	4.0	0.17 " "	0.281	0.302	0.045	
	5.0	0.19 " "	0.314	0.339	0.050	
Morrison (1923)	3.0	0.257–0.286 pounds T.D.N.	0.239–0.266	0.257–0.286		0.047–0.057
	4.0	0.311–0.346 " " " "	0.289–0.321	0.311–0.346		0.054–0.065
	5.0	0.362–0.402 " " " "	0.336–0.373	0.362–0.402		0.060–0.073
	6.0	0.409–0.454 " " " "	0.380–0.422	0.409–0.454		0.067–0.081
	7.0	0.454–0.505 " " " "	0.422–0.469	0.454–0.505		0.074–0.089
Forbes and Kriss (1931)	3.0	0.251 pounds T.D.N.	0.233	0.251		0.038–0.054
	4.0	0.300 " " " "	0.279	0.300		0.045–0.063
	5.0	0.350 " " " "	0.325	0.350		0.050–0.070
	6.0	0.393 " " " "	0.365	0.393		0.056–0.078
	7.0	0.433 " " " "	0.403	0.433		0.061–0.085

* Computed by the writer from the data on page 000, in direct proportion to the live weight, for the purpose of comparison, since in Kellner's standard the requirements for maintenance and for milk production are not given separately.

** Considered by Armsby to be the equivalent of 0.5 pound true protein.

1 Kgm. milk production value is equivalent to 2.800 Therms milk energy. (Hansson)
1 food unit is equivalent to 0.7 Kgm. starch value. (Hansson)
1 food unit is equivalent to 0.75 Kgm. milk production value. (Hansson)
1 Therm metabolizable energy is equivalent to 0.575 Therm net energy for fattening. (Forbes and Kriss)
1 Therm metabolizable energy is equivalent to 0.693 Therm milk energy. (Forbes and Kriss)
1 pound total digestible nutrients is equivalent to 1.616 Therms metabolizable energy. (Forbes and Kriss)
1 pound total digestible nutrients is equivalent to 0.929 Therms net energy for fattening. (Forbes and Kriss)

By the use of these factors such a comparison as suggested above is made in the preceding table, all statements of energy requirements being computed to equivalent quantities of total digestible nutrients, to provide a basis for comparison.[4]

MAX KRISS

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[4] While this review was in press an article by E. Mangold—Der Stärkewert und Andere Futtereinheiten—a separate from Mangold's Handbuch der Ernährung und des Stoffwechsels der Landwirtschaftlichen Nutztiere, third volume, 1931, came to hand. This article covers much the same ground as the foregoing review, and expresses much the same general point of view.—The Author.

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